12th congress

EUROPEAN SOCIETY FOR PHOTOBIOLOGY

ESP 2007

BATH, ENGLAND

1st – 6th September 2007

Programme

and

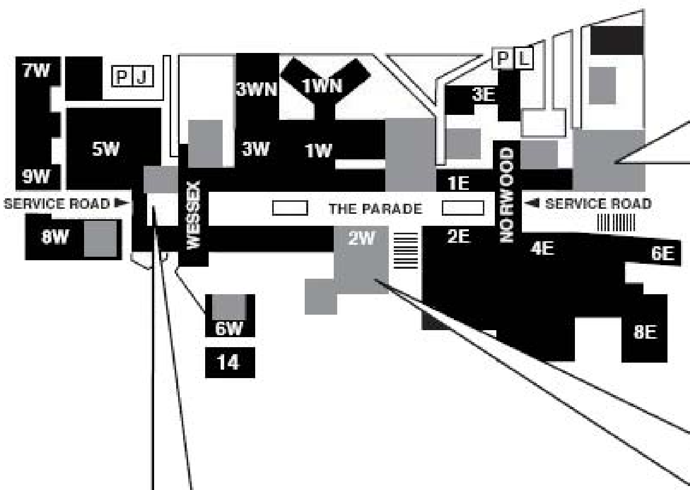
Book of Abstracts

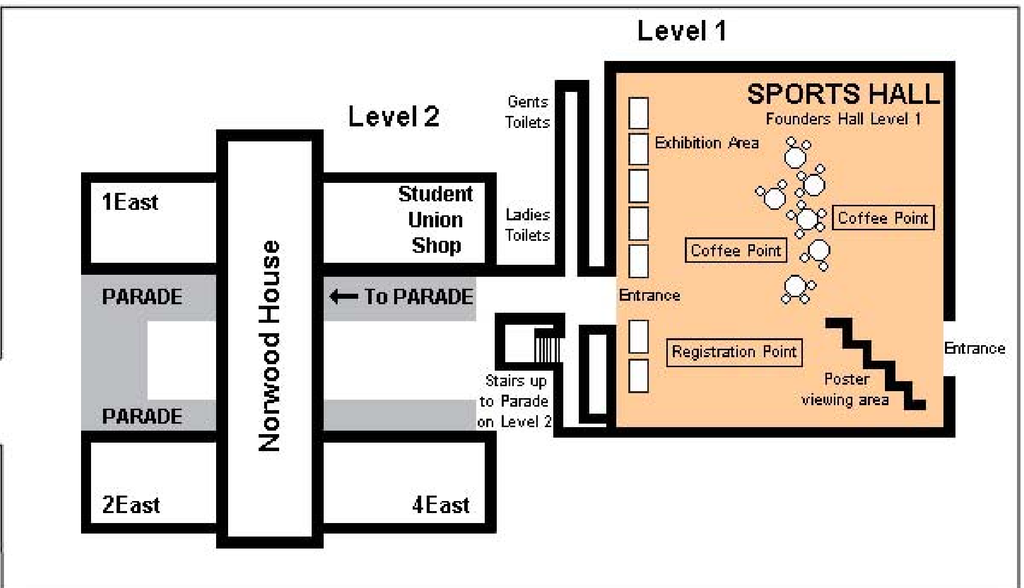
	Saturday 1 Sep	Sunday 2 Sep	Monday 3 Sep	Tuesday 4 Sep	Wednesday 5 Sep	Thursday 6 Sep
8.30		***UH:* Opening Ceremony Welcome Addresses**	***UH:* Photobiology Update** Stephen Bown: Overview of clinical applications of PDT	***UH:* Photobiology Update** Pill-Soon Song: From photochemistry and photobiology to photobiotechnology of plants	***UH:* Photobiology Update** Janet F. Bornman: Impacts of UV-B radiation on biotic and abiotic systems in relation to global climate change	***UH:* Photobiology Update** Leon H.F. Mullenders: DNA repair from past to future
8.45		***UH:*** Young Scientist Award Lecture Virginie Lhiaubet-Vallet: Interactions between drug excited states and proteins or DNA				
9.15		*Coffee Break*	*Coffee Break*	*Coffee Break*	*Coffee Break*	*Coffee Break*
9.45		9.45 – 13.00: **Parallel Symposia** ***UH:*** DNA damage and repair ***8W11:*** ALA-based PDT ***5W23:*** New aspects of photo-immunology: mouse and man ***8W21:*** Instrumentation in photobiology	9.45 – 13.00: **Parallel Symposia** ***UH:*** Vitamin D and solar radiation ***8W11:*** Clinical applications of PDT and PDD ***5W23:*** Oral communications ***8W21:*** Photobiological techniques for environmental monitoring and control	9.45 – 13.00: **Parallel Symposia** ***UH:*** Biological consequences of UV damage ***8W11:*** Biophysics of photosensitization ***5W23:*** A reconstruction of the past UV climatology over Europe for photo-biological studies - Joint with the EU Programme COST 726 ***8W21:*** Photodermatoses In memoriam of Ian Magnus (1920-2006) - Joint with the BPG	9.45 – 13.00: **Parallel Symposia** ***UH:*** Molecular and cellular effects of UV-A ***8W11:*** New approaches to clinical phototherapy ***8W21:*** PDT, light and the immune system	9.45 – 13.00: **Parallel Symposia** ***UH:*** Photodiagnosis and optical techniques for medical diagnosis ***8W11:*** Recent advances in the characterization of photooxidative damage at the biomolecular level ***8W21:*** Blue light regulation in plants and microorganisms
12.00	Excecutive Committee I					
13.00		*Lunch Break*	*Lunch Break*	*Lunch Break*	*Lunch Break*	*Closing*
14.00		***UH:*** Presentation of the European Platform for Photodynamic Medicine (EPPM)				
14.15		***UH:* Photobiology Update** Vivienne E Reeve: Multiple regulatory pathways for photoimmune responsiveness	***SH:* Poster Session I**	14.15 – 17.15: **Parallel Symposia**	***SH:* Poster Session II**	
14.30	Registration					
15.00		15.00 – 18.00: **Parallel Symposia** ***UH:*** Molecular and cellular aspects of PDT		***UH:*** Photochemistry and phototoxicity of drugs ***8W11:*** PDT oral communications		
16.00		***8W11:*** Sun sensitivity, DNA repair and skin cancer ***5W23:*** Photosynthesis: mimicking the natural system for solar energy conversion In honour of James Barber	16.00 – 19.00: **Parallel Symposia** ***UH:*** Drug delivery technologies for PDT	***5W23:*** Photoprotection Joint with the ESPD ***8W21:*** UV and global climate change: effects in aquatic and terrestrial ecosystems - Joint with the ASP	16.00 – 19.00: **Parallel Symposia** ***UH:*** PDT to treat microbial infections *In memoriam* of John D. Spikes (1918-2006)	
17.15		***8W21:*** Bioluminescence: from protein structure to biosensing applications	***8W11:*** Photochemistry and photobiology of carotenoids - Joint with the EPA	**ESP General Assembly**	***8W11:*** Experimental photocarcinogenesis	
18.00			***5W23:*** UV and oxidative stress ***8W21:*** Photoorientation in plants and microorganisms		***8W21:*** Mechanistic and applied approaches to plant response in a changing climate	
19.00	Welcome Reception		PPS Editorial Board Meeting		Executive Committee II	
19.30				Banquet, Medal Awards		

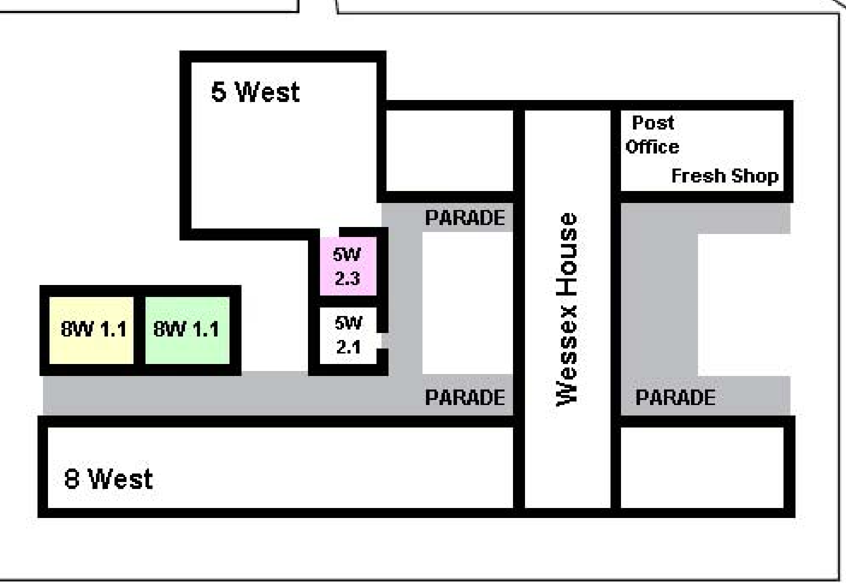
ROOMS:

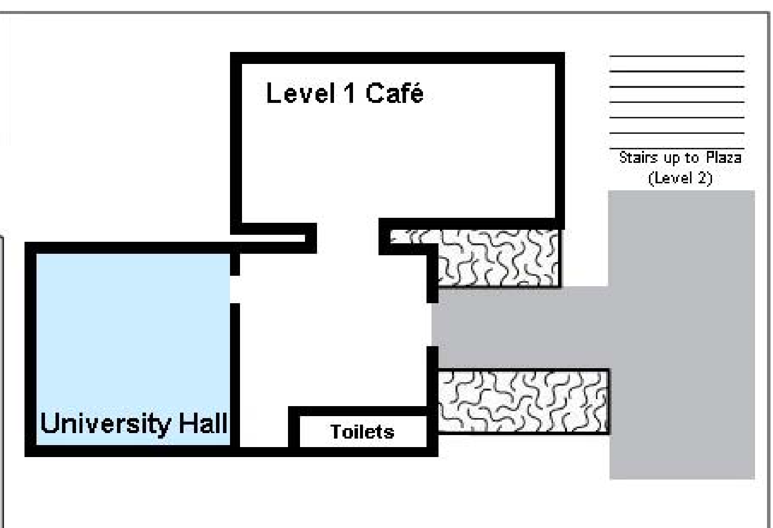
- ***UH:*** *University Hall*
- ***8W11:*** *Room 8W 1.1*
- ***5W23:*** *Room 5W 2.3*
- ***8W21:*** *Room 8W 2.1*
- ***SH:*** *Sports Hall*

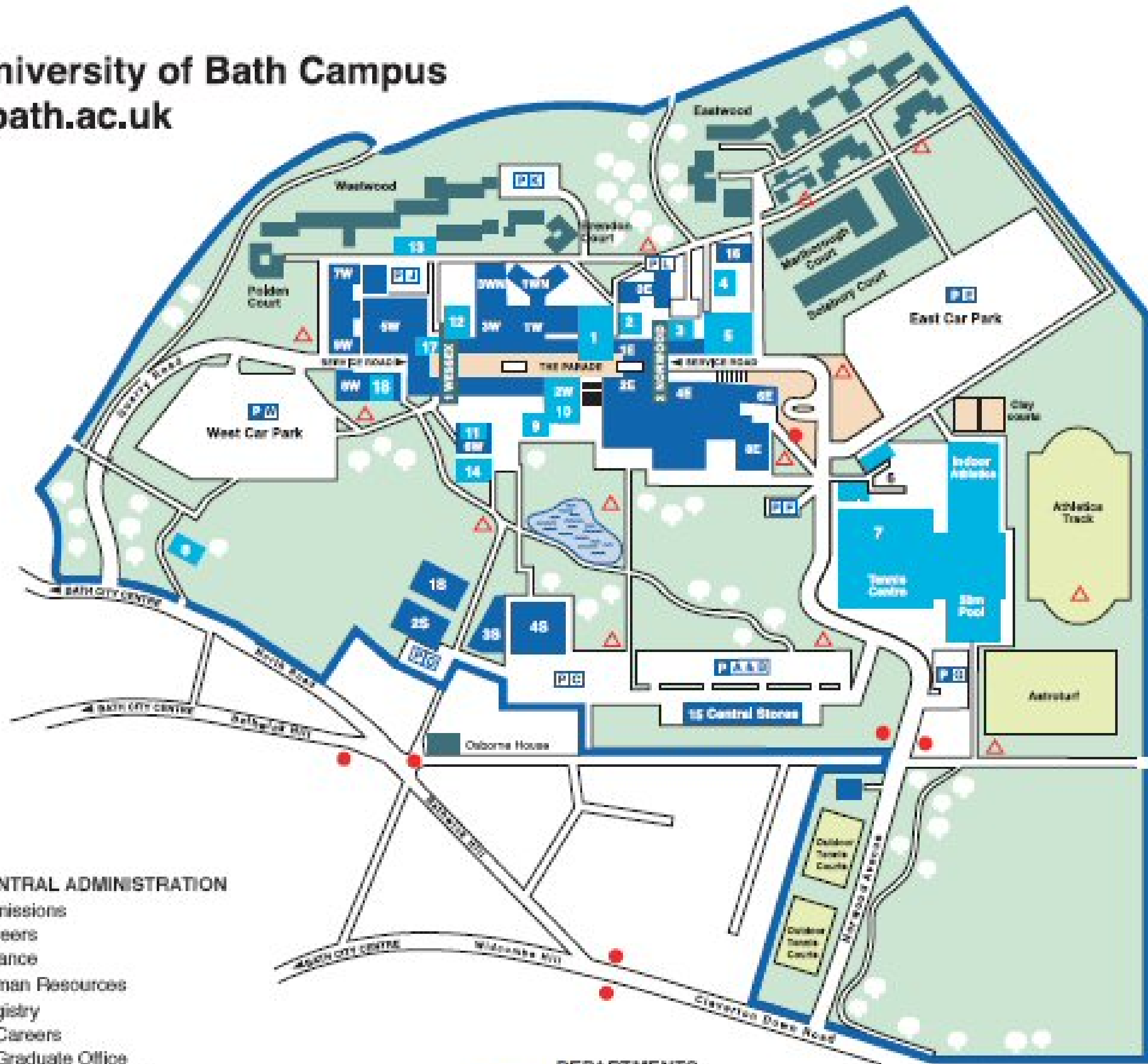
Meeting Rooms and Exhibition and Poster Area
University of Bath











Wessex **CENTRAL ADMINISTRATION**

- Admissions
- Careers
- Finance
- Human Resources
- Registry
  - Careers
  - Graduate Office
  - International Office
  - Student Records and Examinations
  - Undergraduate Admissions
- Security and Enquiries
- Vice-Chancellor

AMENITIES

- 1 Library
- 2 Chaplaincy Centre
- 3 Students Union
- 4 25m Swimming Pool
- 5 Sports Hall
- 6 Arts Barn & Arts Lecture Theatre (ICIA)
- 7 Sports Training Village
- 8 Medical/Dental Centre
- 9 University Hall
- 10 Level 1 Café, Parade Bar, Claverton Rooms Restaurant
- 11 Student Accommodation Office
- 12 Post Office, Banks & Shops
- 13 Accommodation Services Centre
- 14 UBSA
- 15 Central Stores
- 16 Estates
- 17 Student Money Centre
- 18 Print Unit

- ● City Bus Service
- △ Fire Assembly Points
- P Parking: Pay & Display and Permit Holders
- P Parking: Permit Holders only

DEPARTMENTS

- 4E 6E Architecture & Civil Engineering
- 4S Biology & Biochemistry
- 2S BUCS
- 9W Chemical Engineering
- 1S 3S Chemistry
- 1W Computer Science
- 3E Economics & International Development
- 1W 1WN Education (Coach Education)
- 2E 4E Electronic & Electrical Engineering
- 1WN European Studies & Modern Languages
- 8W Management
- 1W Mathematical Sciences
- 4E 6E Mechanical Engineering (Sports Engineering)
- 3S Natural Sciences
- 5W 7W Pharmacy & Pharmacology
- 3W Physics
- 2S Psychology
- NH School for Health
- 3E Social & Policy Sciences
- 6W Sport & Exercise Science

ACCOMMODATION

- Wessex House
- Norwood House
- Polden Court
- Westwood
- Brendon Court
- Eastwood
- Marlborough Court
- Solsbury Court
- Osborne House

Dear Photobiologists,
Dear Friends,

I am delighted to welcome you to Bath on the occasion of the 12th Congress of the European Society for Photobiology.

You will see from the details that follow that the Scientific Organizing Committee have assembled an impressive and varied Scientific Programme that covers as broad an area as practical of the Science of Photobiology and has attracted plenary lecturers, symposium speakers and delegates from every corner of the planet to make this a truly international meeting in keeping with the mission of the ESP as a genuinely global scientific society. As usual there are several joint symposia with societies and groups with common interests including the American Society for Photobiology, the British Photodermatology group, the European Society for Photodermatology, the European Photochemistry Association as well as the EC funded UV climatology consortium COST and these interactions have helped further to diversify the programme.

It is a particular pleasure for me to welcome you all to this University, the University of Bath, which I first joined as a student soon after the institution gained its Royal Charter and which I rejoined a few years ago. The University has grown both in size and stature since those early days to become one of the top ten British Universities. My own department of Pharmacy and Pharmacology, which will host our business meetings, has its origins in Bath in a much earlier era and is proudly celebrating its centenary this year having achieved its place as one of the two or three leading Pharmacy research Departments in the UK.

Importantly, the University is renowned for its warm friendly atmosphere greatly aided by its relatively small size and compact but very green and pleasant campus. We sincerely hope that you can share in this warmth and fully enjoy the science, the convivial atmosphere as well all the attractions of the ancient city of Bath during your stay.

Rex Tyrrell
Local Chairman

Organizing Committee

Francesco Lenci (ITA), Chair and President of the ESP
Rex M. Tyrrell (GBR), Local Chairman
Kristian Berg (NOR), ESP President Elect
Jean Cadet (FRA), Chairman of the 2005 ESP Congress
Francesco Ghetti (ITA), ESP Treasurer
Jacques Piette (BEL), ESP Past President

Scientific Committee

Honnavara Ananthaswamy (USA)
Christophe Bédane (FRA)
Kristian Berg (NOR)
Janet F. Bornman (NZL)
Jean Cadet (FRA)
Piergiacomo Calzavara-Pinton (ITA)
Gianfranco Canti (ITA)
Colin Chignell (USA)
Frank de Grujil (NED)
Benjamin Ehrenberg (ISR)
Francesco Ghetti (ITA)
Neil Gibbs (GBR)
Albert Girotti (USA)
Donat-Peter Häder (GER)
Giulio Jori (ITA)
Irene Kochevar (USA)
Herwig Kostron (AUT)
Kenneth H. Kraemer (USA)
Francesco Lenci (ITA)
Aba Losi (ITA)
Johan Lugtenburg (NED)
Zvi Malik (ISR)
Miguel A. Miranda (SPA)
Patrick Neale (USA)
Allen Oseroff (USA)
Jacques Piette (BEL)
Vivienne Reeve (AUS)
Angelika Rück (GER)
Alain Sarasin (FRA)
Alison Telfer (GBR)
George Truscott (GBR)
Rex M. Tyrrell (GBR)
Vadim Viviani (BRA)
Georges Wagnières (SUI)
Peter Wolf (AUT)
Antony Young (GBR)
Gaetano Zipoli (ITA)

Congress Venue

The scientific part of the meeting will be held at the University of Bath.

University of Bath
Claverton Down
Bath BA2 7AY, UK
Tel: +44(0)1225 386793
Fax: +44(0)1225 383408
http://www.bath.ac.uk/external.html
E-mail: R.M.Tyrrell@bath.ac.uk or prsrmt@bath.ac.uk

Local Organization

Chairman: Professor Rex M. Tyrrell (prsrmt@bath.ac.uk)

Organiser and Co-ordinator: Miss Helen Thame (Tel: +44(0)1225 386850, e-mail: prshct@bath.ac.uk)

Department of Pharmacy and Pharmacology
University of Bath
Claverton Down
Bath BA2 7AY, UK

With organizing support from Prof. R.H. Guy, Dr. S.H. Moss and Dr. C. Pourzand

Official Language

The conference language will be English with no translation facilities available.

Registration

Saturday 1st September	University Hall	14.30 - 18.00
Sunday 2nd September	Founders Sports Hall	09.15 - 14.15
Monday 3rd to Thursday 6th September	Founders Sports Hall	09.15 - 10.15

Congress registration fees

	Before 1 June	Before 15 August	On-site payment
ESP-Members	£280	£350	£385
ESP-Members (Emerging Countries)	£170	£240	£280
ESP-Members (Students)*	£125	£150	£175
Non-ESP-Members**	£400	£470	£500
Accompanying persons		£35	
Social Dinner Tuesday, 4 September 2007		£46	

* Students: Certification letter required.
** For non-ESP members: You may apply for ESP membership at the ESP website www.esp-photobiology.it.

Registration fee includes access to all the scientific sessions and the exhibition/poster/meeting hall, the book of abstracts, coffee breaks and the welcome reception.

ESP-Membership fees for 2007 in EUR

The membership fee includes the subscription to the official journal of the ESP, **Photochemical & Photobiological Sciences (PPS)**, published by the Royal Society of Chemistry (RSC, UK), and available both in electronic and hardcopy versions. Please note that a subscription for more than one year guarantees you a fixed price as well as a reduction on a per-year basis.

	Electronic version	**Electronic plus hardcopy**
1 year	**45** EUR **(students only)**	**136** EUR
2 years	**100** EUR	**270** EUR
4 years	**196** EUR	**536** EUR

Cancellation policy for congress fees and accommodation

Please contact the ESP Treasurer Dr. Francesco Ghetti, CNR Istituto di Biofisica, Area della Ricerca di Pisa, Via G. Moruzzi 1, 56100 Pisa, Italy (Telephone: +39 050 3152764, Fax: +39 050 3152760, e-mail: francesco.ghetti@pi.ibf.cnr.it).
All refunds will be processed after the congress.
Please note that 85% of the registration fee will be refunded, but the social dinner fee will not be reimbursed unless cancellation is received before August 25.
If accomodation booking cancellation is received after the 11th August refunds will only be made at 50 percent of cost. No refunds if cancellation is received after the 27th August.

Location of Scientific Activities

The opening ceremony and the plenary talks will be held in the newly refurbished University Hall. The symposia will be held in the University Hall and 3 modern lecture theatres (8W 1.1, 8W 2.1, 5W 2.3).
All posters, exhibitions, coffee breaks (from Sunday 2 to Thursday 6, 9.15 am to 9.45 am) and main meeting area will be in the Old University Sports Hall (Founders Sports Hall).

Information for presenters

Oral Presentations

Oral presentations will be in the one of the 4 lecture rooms (University Hall, 8W 1.1, 8W 2.1, 5W 2.3).
The presentation times are variable but should strictly adhere to those given in the programme schedule.
Projection will be via PowerPoint using the Windows XP operating system and standard data projectors.
Speakers should check their presentations for compatibility with PowerPoint 2003 for Windows.
All presentations should be pre-loaded **at least half a day in advance**. Speakers should go to room 5W 2.1 to upload and check their presentations which should be provided on a CD or preferably a flash memory stick.
Speakers for the Sunday morning sessions will be given further information on loading of presentations at the reception desk.

Posters

Posters should be prepared in AO portrait format (85 cm width, 120 cm height) since the poster boards are 2m high by 1m wide.
No pins can be used to fix the posters. Self-adhesive Velcro pads will be provided.
The posters can be mounted starting on Sunday 2nd September and must be removed at the latest by 16.00 on Wednesday 5th September when the poster boards will be dismantled. However they can all remain on display throughout this period. There will be two poster sessions (Monday 3rd September and Wednesday 5th September from 14.00 to 15.30).
Each poster has been assigned a number which can be found in the programme book and must be mounted on the poster board with the same number.
The poster session for each poster can also be found in the programme book and presenters should be available for the duration of the corresponding session.

Main Social Events

Welcome and Civic Reception
Saturday 1st September, 19.00 – 20.30 in the Roman Baths and Pump Room complex
Special buses will leave from the University starting at 17.45 and until 18.30. We encourage people to take the earlier buses where possible. Information on this will be posted in the registration area
The reception will be held in the Roman Baths and Pump Room complex (in Bath city centre). There will be drink(s) and nibbles but you will need to make your own arrangements for dinner. We will be formally welcomed by the Chairman of the Local Council during the reception. The Baths are the excavated and largely original leisure/thermal spa area of the Romans. The Pump Rooms were the social centre of the Georgian era playboys.
There will be no coaches back to the University as people are likely to want to return at quite different times.

Conference dinner and Medal Award Ceremony
Tuesday 4 September at 19.30 in the Assembly Rooms
This is for ticket–holders only and will be held in the Assembly Rooms, a masterpiece of Georgian architecture, situated close to the Bath city centre.
Buses will leave from the University at 19.00, and return to the University at 23.30. Information on this will be posted in the University Hall entrance and Sports Hall.
During the Banquet the ESP Medals, for "Excellence in Photobiological Research and Dedication to the ESP", and the Young Scientist Medal will be awarded. The recipient of the Young Scientist Medal, Virginie Lhiaubet-Vallet, will give the Congress opening lecture.

Business Meetings (all to be held in building 7W room 2.6)

Saturday 1st September, 12.00-15.30	ESP Executive Committee 2005-2007
Monday 3rd September, 19.00-20.00	Editorial Board, Photochemical and Photobiological Sciences Photobiology section
Wednesday 5th September, 19.00-20.00	ESP Executive Committee 2007-2009
Thursday 6th September, 09.30-14.00	Ownership Board, Photochemical and Photobiological Sciences

The ESP General Assembly will be held on
Tuesday 4th September, from 17.30 to 18.30, in the University Hall

Meals

Vouchers will be distributed at registration to those who have pre-booked breakfast (included in University accommodation charges) and lunches. Both breakfast and lunch (with vouchers) will be served in the level 1 café immediately adjacent to the University Hall.
It is envisaged that most people will want to go in to town for dinner but evening meals will also be available on the campus. In addition there are snack bars, coffee bars and a pub on site as well as food shops, banks and post-office.

Computer access and wireless networking

A large number of computers are available in the library for use by conference participants. However every participant who wishes to use this service for the internet and to check e-mail accounts will need to have registered in advance (as posted on the conference web-site and in individual mailings to registered participants) in order that a username and password can be issued. A £5 charge is made by the University to all users for the duration of the conference.
Personal laptops can also be used but you will also need to have registered in advance and paid the £5 fee. Configuration instructions will be given with the passwords. Please note that although wireless access is available in many common areas of the University, there is insufficient signal in most of the University

residences. Ethernet connections are not available to conference participants in the residences and modems cannot be used on campus through the phone lines.
Only fully paid-up registrants who have sent the computer access form and invited speakers who have registered and sent the form will have been added to the list for issue of usernames (and our computer centre will not issue usernames unless we tell them well in advance so we cannot add names on site).

Car Parking

Car parking places are quite rare and expensive in Bath. For those staying on campus, we will be able to provide permits on site at a cost of £5 for the whole conference period.

Programme for accompanying persons

The accompanying persons programme will be provided at registration

List of sponsors (on printing date)

City of Bath

Perceptive Instruments http://www.perceptive.co.uk

Charles River Laboratories http://www.criver.com/

Therakos - Immune Cell Therapy http://www.therakos.com/

DSM http://www.dsm.com/en_US/html/dnp/home_dnp.htm

Prizmatix http://www.prizmatix.com/

Vifor (International) Inc. http://www.vifor.com

Bruker-Biospin Ltd

British Photodermatology Group

List of Exhibitors

Royal Society of Chemistry, Great Britain http://www.rsc.org/pps
Publishers of Photochemical and Photobiological Sciences

Perceptive Instruments http://www.perceptive.co.uk

Bentham http://www.bentham.co.uk/

Optronic Laboratories http://www.olinet.com/

Blackwell Publishing http://www.blackwellpublishing.com/

Skye Instruments http://www.skyeinstruments.com/

Additional information provided by the University

We hope that your stay with us will be comfortable & enjoyable. You may find the following information helpful.

Accommodation Services Centre (ASC)
The ASC is located to the front of the Westwood Accommodation our staffs are here to help ensure you have an enjoyable and comfortable stay. You can visit the office or call us on extension 5883 from an internal phone or 01225 385883 for any enquiries you may have. The opening hours are:
Monday to Friday 8.30am to 10.00pm
Saturday and Sunday 9.00am to 10.00pm

Emergencies
The University Security Office is located on the main Parade opposite the Fresh Shop and is manned 24 hours a day. In an emergency they can be contacted from any University phone by dialling 666, for non-emergencies they can be contacted on extension 5349, or 01225 385349.

Taxis
There are a number of local taxi companies:
Abbey Taxis 01225 444444
Bath Taxis 01225 447777
City Taxis 01225 840333

Campus facilities

Level One and Wessex Restaurants
Meals in both restaurants need to be pre-booked prior to arrival by your event organiser who will provide you with full details. If your organiser has booked meals then identification must be shown.

The Parade Bar
Is located on The Parade Level 2 and offers a wide selection of food and snacks throughout the day, is fully licensed and is open as follows:
Monday to Friday 11.00am to 11.00pm
Saturday 5.00pm to 11.00pm
Sunday 5.00pm to 10.30pm

Dolche Vita
Is an espresso bar located on The Parade Level 2 next to the Fresh Shop and is open for breakfast and light snacks, speciality tea's and coffee's and is open:
Monday to Friday 8.00am and 4.00pm
Saturday and Sunday 9.00am to 4.00pm

Munchies
Located within the Students' Union Norwood House Level 2, serving a wide variety of foods including burgers, jacket potatoes, sandwiches and pizzas, is open Monday to Friday and some weekends (please check venue for opening hours).
Monday to Friday 8.00am to 3.30pm

Shops, Post Office & Newsagents
The Fresh Shop and Fresh Oriental are located on the Parade at the foot of Wessex House and are stocked with everyday groceries and magazines. Fresh also includes a Post Office. On level 2 (parade level) of Norwood House can be found the Student Union Shop and the campus branch of the bookshop

Waterstone's. Post collection boxes are located outside the Fresh Shop and the 3 East Building. The shops are open:

Fresh:	Monday to Friday 7.30am to 7.00pm; Saturday & Sunday 9.00am to 4.00pm
Post Office:	Monday to Friday 9.00am to 5.30pm (Closed for lunch 1pm to 2pm)
Fresh Oriental:	Monday to Friday 11.00am to 5.00pm
SU Shop:	Monday to Friday 8.30am to 5.30pm
Waterstone's:	Monday to Friday 9.00am to 5.00pm

Banks

There are banks on the campus with cash machines. All are located on the Parade just along from the Fresh Shop. There is also an Alliance and Leicester machine located in the Students' Union (Level 2 Norwood House). Opening hours are as follows:

Barclays Bank	Monday to Friday 10.30am to 2.30pm
NatWest Bank	Monday to Friday 10.00am to 3.00pm

Launderette

There are launderettes on campus, these can be used by delegates staying in University accommodation. There are laundrettes in Eastwood, Westwood and Norwood. The Eastwood Laundrette is located next to the Eastwood A housekeepers office, the Westwood laundrette is next to the Accommodation Services Centre, and the Norwood Laundrette is on level 2 of Norwood House opposite Munchies.

Transport

There is a regular bus service from the University bus stop to the city centre. The bright orange bus, which is a number 18 or 418, is the most frequent and quickest bus service to and from the city centre. The train station is a 2 min walk from the bus station in the city centre. The bright orange operates:
Monday to Friday runs from 7.40 am until 10.55 pm approximately every 15mins
Saturday runs from 7.40 am until 10.55 pm approximately every 20mins
Sunday runs from 8.55 am until 10.55 pm and approximately every 30mins

Sports Facilities

The University of Bath is home to some of the best athletes and some of the best sporting facilities in the country. It caters for elite athletes and people of all abilities alike, and many of its facilities are open to the public. The University has two swimming pools, a 50 metre pool which is located in the Sports Training Village (STV) and a 25m pool which is behind Founders Hall. These are both available for use by members of the public at certain times, for further information and timings please contact STV reception on 01225 386339.

All opening times and details are subject to change; information was correct at time of going to print 31 July 2007

All Authors are kindly requested to carefully check for mistakes (Authors' names and addresses, title and body of the abstract) and notify them to Francesco Ghetti (francesco.ghetti@pi.ibf.cnr.it) for the final version of the Programme and Book of Abstracts that will be posted on the web after the Congress.

Saturday, September 1, Afternoon — University Hall Foyer

14.30
18.00 **Registration**

Saturday, September 1, Evening — Roman Bath and Pump Rooms

19.00
20.30 **Welcome Reception**

Sunday, September 2, Morning — University Hall

08.30 **Opening Ceremony – Welcome Addresses**

08.45 **PL101** **Young Scientist Award Lecture**
Interactions between drug excited states and proteins or DNA
Virginie Lhiaubet-Vallet (Valencia, ESP) *introduced by Kristian Berg (Oslo, NOR)*

09.15 **Coffee Break**

09.45 **DNA damage and repair**
Chair: Jean Cadet (Grenoble, FRA)

09.45 **IL102** **Absorption of UV radiation by DNA helices: collective effects and time-resolved studies**
D. Markovitsi, D. Onidas, T. Gustavsson, F. Talbot, S. Marguet, E. Lazzarotto (Gif-sur-Yvette, FRA)

10.15 **IL103** **Photochemistry of 5-halouracil-containing DNA**
Hiroshi Sugiyama (Kyoto, JPN)

10.45 **IL104** **Structural investigation of cyclobutane pyrimidine dimers in complex with DNA photolyase and RNA polymerase II**
Thomas Carell (Munich, GER)

11.15 **IL105** **Specific features of the formation and repair of far-UV induced dimeric photoproducts at the four bipyrimidine dinucleotides within isolated and cellular DNA**
Thierry Douki[1], Stephane Mouret[1], Evelyne Sage[2], Fabien Joux[3], Sabine Matallana-Surget[3], Jarah A. Meador[3], Jean Cadet[1] ([1]Grenoble, [2]Orsay, [3]Banyuls-sur-Mer, FRA)

11.45 **OC106** **Induction and repair of UV-induced DNA damage in *Deinococcus radiodurans***
Petra Rettberg[1], Ulrike Pogoda de la Vega[1], Thierry Douki[2], Jean Cadet[2], Günther Reitz[1] ([1]Köln, GER; [2]Grenoble, FRA)

12.00 **OC107** **Towards time-resolving electron transfer between different tryptophan residues during DNA photolyase activation**
Martin Byrdin[1], Andre P.M. Eker[2], Agathe Espagne[1], Sandrine Villette[1], Klaus Brettel[1] ([1]Gif-sur-Yvette, FRA; [2]Rotterdam, NED)

Sunday, September 2, Morning — Room 8W 1.1

09.45 **ALA-based PDT**
Chair: Zvi Malik (Ramat Gan, ISR)

09.45 **IL108** **ALA-PDT cell death: real time imaging of subcellular processes**
Zvi Malik, Dana Darvish, Aryeh Weiss (Ramat Gan, ISR)

10.15 **IL109** **Mechanisms underlying light fractionated PDT using porphyrin precursors**
Henriette S. de Bruijn, Ellen R. M de Haas, Henricus J. C. M. Sterenborg, H A Martino Neumann, Dominic J Robinson (Rotterdam, NED)

10.45 **IL110** **Detection of urinary bladder cancer with flow cytometry and hexaminolevulinate in urine samples**
Beata Čunderlíková, Rolf Wahlqvist, Aasmund Berner, Vlada Vasovič, Trond Warloe, Jahn M. Nesland, Qian Peng (Oslo, NOR)

11.15 **OC111** **Photodynamic therapy using topically applied hypericin: comparative effect with methyl-aminolevulinic acid on UV induced skin tumors**
Annelies Boiy, Rik Roelandts, Peter de Witte (Leuven, BEL)

11.30 **OC112** **Orthotopic animal models for oncologic photodynamic therapy and photodiagnosis**
Marie Ange D'Hallewin, Lina Bezdetnaya, Henri Pierre Lassalle, François Guillemin (Vandoeuvre-les-Nancy, FRA)

11.45 **OC113** **Liposomes of aminolevulinic acid and aminolevulinic acid-esters for photodynamic therapy**
Alcira Batlle[1], Gabriela DiVenosa[1], Adriana Casas[1], Laura Hermida[1], Haydée Fukuda[1], María Victoria Defain[1], Alexander MacRobert[1,2] ([1]Buenos Aires, ARG; [2]London, GBR)

12.00 **OC114** **Novel 5-aminolaevulinic acid peptide prodrugs for photodynamic therapy: synthesis, characterisation, and cellular accumulation studies**
Francesca Giuntini[1], Ian M. Eggleston[1], Ludovic Bourre[2], Alexander J. MacRobert[2], Michael Wilson[2] ([1]Bath, [2]London, GBR)

12.15 **OC115** **Histamine, nitric oxide and prostaglandin E2 are released following topical ALA-PDT of human skin. 'Real-time' investigation using intradermal microdialysis**
Rebecca Brooke[1], Meneka Sidhu[1], Rachel Watson[1], Martin Church[2], Peter Friedmann[2], Geraldine Clough[2], Lesley Rhodes[1] ([1]Manchester, [2]Southampton, GBR)

12.30 **OC116** **Enhanced porphyrin accumulation using peptide derivatives of 5-aminolaevulinic acid for photodynamic therapy**
Ludovic Bourre[1], Francesca Giuntini[2], Ian M. Eggleston[2], Michael Wilson[1], Alexander J. MacRobert[1] ([1]London, [2]Bath, GBR)

Sunday, September 2, Morning — Room 5W 2.3

09.45 **New aspects of photoimmunology: mouse and man**
Chairs: Vivienne Reeve (Sydney, AUS), Prue Hart (Perth, AUS)

09.45 **IL117** **Polymorphic Light Eruption is associated with abnormalities in keratinocyte responses to UVR**
Susan Lesley Walker (London, GBR)

10.10 **IL118** **Unbalanced immune reactions to UV-B irradiation in Polymorphic Light Eruption**
A Soe Janssens, Stan Pavel, Frank R de Gruijl (Leiden, NED)

10.35 **IL119** **Regulation of murine asthma models by UV radiation of skin**
Prue H Hart, Jacqueline P McGlade, Shelley Gorman, John J Finlay-Jones (Perth, AUS)

11.00 **IL120** **The role of UV-induced chemokines in modulating immune responses**
Scott N. Byrne[1], Alberto Y. Limon-Flores[2], Stephen E. Ullrich[2] ([1]Sydney, AUS; [2]Houston TX, USA)

11.25 **OC121** **Chronotherapy - Circadian rhythm, the human immune response and treatment of disease**
Joan Elizabeth Roberts (New York NY, USA)

11.40 **OC122** **UVB radiation and vitamin D influence the neonatal skin immune system and can have long term implications**
Gregory M Woods, Roslyn C Malley, Heather M McGee, H Konrad Muller (Hobart, AUS)

11.55 **OC123** **Cis-urocanic acid initiates gene transcription independently of the serotonin 5HT2A receptor in primary human keratinocytes**
Kazuyo Kaneko[1], Ulli Smetana-Just[1], Mary Matsui[2], Antony R Young[1], Susan John[1], Mary Norval[3], Susan L Walker[1] ([1]London, [3]Edinburgh, GBR; [2]Melville NY, USA)

12.10 **OC124** **Modulation of photoimmune suppression by exogenous or endogenous melatonin**
Kishen Lachireddy, Diane Domanski, Vivienne E Reeve (Sydney, AUS)

12.25 **OC125** **Upregulation of pro- and anti-inflammatory lipid mediators during the sunburn response in human skin. Candidates for mediation and regulation of UVR-induced neutrophil chemotaxis**
A. Haylett[1], M. Masoodi[2], M. Brownrigg[1], K. Gledhill[2], D.J. Tobin[2], A.J. Thody[3], A. Nicolaou[2], L.E. Rhodes[1] ([1]Manchester, [2]Bradford, [3]Newcastle, GBR)

12.40 **OC126** **A characterisation of iNKT cells in mouse skin**
Kirsten J.L. Hammond, Carling Y-Y. Chan, Gary M. Halliday (Sydney, AUS)

Sunday, September 2, Morning — Room 8W 2.1

09.45 **Instrumentation in photobiology**
Chair: Angelika Rück (Ulm, GER)

09.45 **IL127** **TCSPC fluorescence lifetime imaging in photobiology**
Wolfgang Becker, Axel Bergmann (Berlin, GER)

10.15 **IL128** **New Total Internal Reflection (TIR) techniques for live cell imaging**
Herbert Schneckenburger[1], Michael Wagner[1], Petra Weber[1], Thomas Bruns[1], Brigitte Angres[2], Heiko Steuer[2], Wolfgang S.L. Strauss[3] ([1]Aalen, [2]Reutlingen, [3]Ulm, GER)

10.45 **IL129** **Time-resolved fluorescence imaging in the life sciences**
Klaus Suhling (London, GBR)

11.15 **IL130** **Multiwavelength fluorescence lifetime imaging (SLIM) in photobiology**
Angelika Rueck, Frank Dolp, Björn v. Einem, Cornelia Steinmetz, Christine v. Arnim, Rudolf Steiner (Ulm, GER)

11.45 **IL131** **Multiphoton tomography in medicine using femtosecond lasers**
Karsten König[1], Iris Riemann[1], Frank Stracke[1], Aisada Uchugonova[1], Rainer Bückle[2], Ronan Le Harzic[1,2], Katja Schenke-Layland[3], Martin Kaatz[2], Peter Elsner[2] ([1]St. Ingbert, [2]Jena, GER; [3]Los Angeles, USA)

12.15 **OC132** **Measurement and assessment of circadian effective radiation of natural and artificial sources**
Dieter Kockott[1], Helmut Piazena[2], Rüdiger Sippel[3] ([1]Hanau, [2]Berlin, [3]Fröndenberg, GER)

12.30 **OC133** **Instrumentation for selective fluorescence detection from deeper layers of the tissue**
Jonas Venius, Ricardas Rotomskis (Vilnius, LTU)

12.45 **OC134** **Real-time evaluation of tissue properties for feed-back dosimetry in interstitial photodynamic therapy**
Johan Axelsson, Ann Johansson, Johannes Swartling, Sara Pålsson, Thomas Johansson, Niels Bendsoe, Katarina Svanberg, Sune Svanberg, Stefan Andersson-Engels (Lund, SWE)

Sunday, September 2, Afternoon — University Hall

14.00 **Presentation of the European Platform for Photodynamic Medicine (EPPM)**
Keyvan Moghissi (York, GBR)

14.15 **PL135** **Photobiology Update**
Multiple regulatory pathways for photoimmune responsiveness
Vivienne E Reeve (Sydney, AUS) *introduced by Rex Tyrrell (Bath, GBR)*

15.00 Molecular and cellular aspects of PDT

Chair: Jacques Piette (Liège, BEL)

15.00 **IL136** **PDT effects on the tumor microenvironment: growing evidence for combined modality approaches**
Charles J. Gomer (Los Angeles CA, USA)

15.30 **IL137** **Molecular response based combinations with PDT**
T. Hasan, N. Solban, S.K. Chang, I. Rizvi, T. Stepinac, A. Liang, Z. Mai, H. Athar (Boston MA, USA)

16.00 **IL138** **Molecular effectors and modulators of cell death induced by PDT**
Michael Dewaele[1], Esther Buytaert[1], Sofie Van Kelst[1], Wim Martinet[2], Jean-Yves Matroule[3], Jacques Piette[3], Patrizia Agostinis[1] ([1]Leuven, [2]Antwerp, [3]Liège, BEL)

16.30 **IL139** **NF-kB is a central element in the fate of cancer cells treated by photodynamic therapy**
Jacques G Piette, Cédric Volanti, Sébastien Bontems, Jean-Yves Matroule (Liège, BEL)

17.00 **OC140** **The effects of iron on protoporphyrin IX accumulation, cellular damage (DNA damage) and cellular viability in aminolaevulinic acid and methyl aminolaevulinate photodynamic therapy**
Andrew Pye, Alison Curnow (Truro, GBR)

17.15 **OC141** **Intracellular and intratissular distribution of Foscan modulates apoptosis induced by photodynamic therapy**
Sophie Marchal, Aurélie François, Julie Garrier, Aude Bressenot, François Guillemin, Lina Bezdetnaya (Nancy, FRA)

17.30 **OC142** **Hypoxia inducible factor as a molecular marker of response to photodynamic therapy with silicon phthalocyanine Pc 4**
Elma D. Baron, Diana Santo Domingo, Andrew Hsia, Nancy L. Oleinick, Valdir Colussi, Kevin D. Cooper (Cleveland OH, USA)

17.45 **OC143** **Inhibition of EGFR-tyrosine kinase activity by photodynamic therapy**
Anette Weyergang, Pål Kristian Selbo, Kristian Berg (Oslo, NOR)

18.00 **OC144** **Inter- and intracellular signaling processes involved in PDT effect on neurons and glial cells**
Anatoly B. Uzdensky (Rostov-on-Don, RUS)

Sunday, September 2, Afternoon — Room 8W 1.1

15.00 **Sun sensitivity, DNA repair and skin cancer**
Chair: Kenneth H. Kraemer (Bethesda MD, USA)

15.00 **IL145** **The photodermatoses - non-carcinogenic responses to ultraviolet irradiation in man**
John L M Hawk (London, GBR)

15.30 **OC146** **UV-exposure and skin-cancer: global variations in sensitivity and scenarios for the 21st century**
Arjan van Dijk, Harry Slaper, Harm van Wijnen (Bilthoven, NED)

15.50 **IL147** **Xeroderma Pigmentosum, when the guardian of the gene pool goes on strike**
Kenneth H Kraemer[1], Jennifer Boyle[2], Takahiro Ueda[1], Kyu Seon Oh[1], Kyoko Imoto[3], Deborah Tamura[1], Sikandar G. Khan[1], John J.DiGiovanna[1] ([1]Bethesda MD, USA; [2]London, GBR; [3]Nara, JPN)

16.20 **IL148** **Polymorphisms in DNA repair genes and cancer risk in the normal population**
Steffen Emmert, Petra Laspe, Lars Boeckmann, Christiane Kuschal, Kai-Martin Thoms, Sandra Blankenburg, Karolin Zachmann, Ullrich Krueger, Holger Haenssle (Goettingen, GER)

16.50 **IL149** **Large scale deletions of the mitochondrial DNA are associated with increased oxidative stress and altered cellular function in skin fibroblasts**
Marc Majora, Tanja Maresch, Peter Schröder, Jean Krutmann (Düsseldorf, GER)

17.20 **OC150** **hOGG1 protein and gene are expressed more abundantly in the superficial than basal layer of human skin epidermis**
Arash Javeri[1], Xiao Xuan Huang[1], Françoise Bernerd[2], Rebecca S. Mason[1], Gary M. Halliday[1] ([1]Sydney, AUS; [2]Clichy, FRA)

17.40 **P605** **The GTPase RhoB, a UVB-responsive protein, regulates p53-independent apoptotic pathways in human normal keratinocytes**
Bruno Canguilhem, Anne Pradines, Marie Charvéron, Gilles Favre (Toulouse, FRA)

Sunday, September 2, Afternoon — Room 5W 2.3

15.00 **Photosynthesis: mimicking the natural system for solar energy conversion**
In honour of James Barber
Chair: Alison Telfer (London, GBR)

Time	Code	Presentation
15.00		**Introductory remarks** Alison Telfer (London, GBR)
15.20	**IL151**	**Molecular solar energy conversion: lessons from photosynthesis** James Durrant (London, GBR)
15.50	**IL152**	**Patterning light harvesting complexes onto self-assembled monolayers using photolithography** Christopher Neil Hunter (Sheffield, GBR)
16.20	**OC153**	**Photosynthetic complexes revealed by single particle analysis** Jon Nield (London, GBR)
16.40	**IL154**	**Variations in the organisation of chlorophyll protein complexes in oxyphotobacteria to exploit different ecological niches** Thomas S Bibby (Southampton, GBR)
17.10	**OC155**	**Carotenoid molecules in Photosystem II** Bruno Robert (Gif-sur-Yvette, FRA)
17.30	**IL156**	**Photosystem II in the unusual chlorophyll d dominated cyanobacterium, *Acaryochloris marina*** Alison Telfer (London, GBR)

Sunday, September 2, Afternoon — Room 8W 2.1

15.00 **Bioluminescence: from protein structure to biosensing applications**
Chair: Vadim Viviani (Sorocaba, BRA)

15.00 **IL157** **The structural origin, biological function and biosensing applications of pH-sensitivity in beetle luciferases**
Vadim R. Viviani[1], F. G. C. Arnoldi[2], A. J. Silva Neto[2], Y. Ohmiya[3] ([1]Sorocaba SP, [2]Rio Claro, BRA; [3]Osaka, JPN)

15.30 **IL158** **Structural insights into bioluminescent mechanism of calcium-regulated photoproteins**
Eugene S. Vysotski[1,2], John Lee[2] ([1]Krasnoyarsk, RUS; [2]Athens GA, USA)

16.00 **IL159** **Basis and application of ostracod bioluminescence**
Yoshihiro Ohmiya[1], Chun Wu[2] ([1]Sapporo, [2]Ikeda, JPN)

16.30 **IL160** **Multicolor reporter gene technology for new multiplexed bioluminescent cell-based assays**
Elisa Michelini[1], Luca Cevenini[1], Laura Mezzanotte[1], Danielle Ablamsky[2], Tara Southworth[2], Bruce Branchini[2], Aldo Roda[1] ([1]Bologna, ITA; [2]New London CT, USA)

17.00 **OC161** **Bioluminescent enzyme-based biosensors**
Valentina A.Kratasyuk, Elena N.Esimbekova (Krasnoyarsk, RUS)

17.15 **OC162** **Studies on the alteration in color of bioluminescence arising from *Vibrio fischeri* Y1**
Hajime Karatani, Takashi Osaki, Masashi Yasui, Shoji Matsumoto, Shogo Ohta (Kyoto, JPN)

17.30 **OC163** **Energy conversion in bioluminescent reactions**
Nadya S.Kudryasheva (Krasnoyarsk, RUS)

Monday, September 3, Morning — University Hall

08.30 **PL201** **Photobiology Update**
Overview of clinical applications of PDT
Stephen Bown (London, GBR) *introduced by Jacques Piette (Liège, BEL)*

09.15 **Coffee Break**

9.45 **Vitamin D and solar radiation**
Chair: Antony Young (London, GBR)

9.45 **Introductory remarks**
Antony Young (London, GBR)

9.50 **IL202** **The relationship between erythemal and vitamin D solar exposure: spectra and doses**
Robert M. Sayre[1,2], John C. Dowdy[1] ([1]Cordova TN, [2]Memphis TN, USA)

10.20 **IL203** **The relationship between exposure of ultraviolet radiation and vitamin D status**
Ola Engelsen (Tromso, NOR)

10.50 **IL204** **Solar UV-radiation, skin cancer and vitamin D: how much sunlight do we need?**
Jörg Reichrath, Bernd Nürnberg, Wolfgang Tilgen (Homburg, GER)

11.20 **IL205** **Solar UVR, vitamin D and prostate cancer: how strong is the relationship?**
Richard C Strange (Stoke-on-Trent, GBR)

11.50 **OC206** **Sun beds and cod liver oil as vitamin D sources**
Johan Moan, Alina Carmen Porojnicu (Oslo, NOR)

12.05 **OC207** **Does casual exposure to UK sunlight provide sufficient vitamin D levels?**
Marie T Durkin, Richard Kift, Lucy V Bunhill, Donald Allan, Jacqueline L Berry, Ann R Webb, Lesley E Rhodes (Manchester, GBR)

12.20 **OC208** **Dose dependent effect of sunbed radiation on cutaneous vitamin D synthesis in humans, a randomized controlled trial**
Elisabeth Thieden, Henrik L. Jørgensen, Niklas Rye Jørgensen, Peter A Philipsen, Hans Chr. Wulf (Copenhagen, DEN)

12.35 **Closing remarks and general discussion**
Antony Young (London, GBR)

Monday, September 3, Morning — Room 8W 1.1

9.45 **Clinical applications of PDT and PDD**
Chair: Herwig Kostron (Innsbruck, AUT)

9.45 **IL209** **ALA and its clinical impact, from bench to bedside**
Barbara Krammer (Salzburg, AUT)

10.05 **OC210** **Molecular signaling in cancer cells after PDT and its clinical relevance**
Charles Gomer (Los Angeles CA, USA)

10.25 **IL211** **Current overview of clinical photodynamic application in neurosurgery**
Sadao Kaneko (Sapporo, JPN)

10.55 **OC212** **Photodynamic therapy and fluorescent diagnostics in clinical settings of Russian Cancer Research Center**
E.G. Vakulovskaya, B.K. Poddybny, Y.P. Kuvshinov, G.V. Ungiadze, V.B. Karakhan, V.V. Kuznecov, V.V. Ashmarov (Moscow, RUS)

11.25 **IL213** **PDT - Registered and future indications in dermatology**
Alexis Sidoroff (Innsbruck, AUT)

11.45 **OC214** **PDT for cholangiocarcinoma: review and personal experience**
Steve Pereira (London, GBR)

12.05 **IL215** **25 years of PDT in neurosurgery: a review**
Herwig Kostron (Innsbruck, AUT)

12.20 **OC216** **Cancer therapy using Vascular-Targeted Photodynamic therapy (VTP) with TOOKAD**
Yoram Salomon, Avigdor Scherz (Rehovot, ISR)

12.40 **OC217** **Cellular and molecular effects of a new chlorine-based photosensitizer in human cancer cells after light exposure**
Heinrich Walt (Zurich, SUI)

12.55 **OC218** **Monitoring ALA and mTHPC-PDT during intraluminal and interstitial PDT**
Bastiaan Kruijt, Angelique van der Ploeg-van den Heuvel, Slavka Kascakova, Arjen Amelink, Henricus. J.C.M. Sterenborg, Dominic J. Robinson (Rotterdam, NED)

Monday, September 3, Morning — Room 5W 2.3

9.45 **Oral communications**
Chair: Jean Cadet (Grenoble, FRA)

9.45 **OC219** **Determination of an action spectrum for the suppression of cutaneous recall immune responses in humans**
Yasmin J Renwick, Tai A Phan, Gary M Halliday, Diona L Damian (Sydney, AUS)

10.00 **OC220** **Topically applied vitamin D activates regulatory T cells in naive mice**
Shelley Gorman, Alexandra Kuritzky, Melinda Judge, Prue Hart (Perth, AUS)

10.15 **OC221** **The effect of UV on effector and memory T cells**
Sabita Rana, Scott N Byrne, Linda J Macdonald, Gary M Halliday (Sydney, AUS)

10.30 **OC222** **Bathochromically shifted hypericin derivatives: photosensitizing properties**
Mieke Roelants[1], Heinz Falk[2], Bernd Lackner[2], Mario Waser[2], Peter A.M. de Witte[1] ([1]Leuven, BEL; [2]Linz, AUT)

10.45 **OC223** **Photosensitised reactions by photoinitiators used for curing dental polymer materials**
Terje Christensen[1,2], Else Morisbak[1], Inger Sofie Dragland[1], Hanne Hjorth Tønnesen[3], Ellen M. Bruzell[1] ([1]Haslum, [2]Østerås, [3]Oslo, NOR)

11.00 **OC224** **Photoactivated therapeutic metal complexes**
Fiona S Mackay[1], Julie A Woods[2], Patrick J Bednarski[3], Pavla Heringova[4], Jana Kasparkova[4], Viktor Brabec[4], Harry Moseley[2], James Ferguson[2], Peter J Sadler[1] ([1]Edinburgh, [2]Dundee, GBR; [3]Greiswald, GER; [4]Brno, CZE)

11.15 **OC225** **Effect of aggregation on photolysis of Merocyanine 540**
Elena A. Kozhinova, Andrei M. Tikhomirov, Alla A. Kyagova, Alexander Ya. Potapenko (Moscow, RUS)

11.30 **OC226** **Influence of lycopene on UVR-induced erythema and mitochondrial DNA damage in human skin**
Muneeza Rizwan[1], Andrew Harbottle[2], Isabel Rodriguez-Blanco[1], Rachel Watson[1], Mark Birch-Machin[2], Lesley Elisabeth Rhodes[1] ([1]Manchester, [2]Newcastle upon Tyne, GBR)

11.45 **OC227** **Fluorescence dynamics of UV-B sunscreens**
Thomas M. Nordlund[1], Rajagopal Krishnan[2] ([1]Birmingham AL, [2]San Francisco CA, USA)

12.00 **OC228** ***In vivo* relevance for photoprotection by the vitamin D rapid response pathway**
Katie M Dixon[1], Shivashni S Deo[1], Anthony W Norman[2], June E Bishop[2], Gary M Halliday[1], Vivienne E Reeve[1], Rebecca S Mason[1] ([1]Sydney, AUS; [2]Riverside CA, USA)

12.15 **OC229** **Is the seasonal variation of cancer prognosis related to photosynthesis of vitamin D?**
Alina Carmen Porojnicu, Johan Moan (Oslo, NOR)

12.30 **OC230** **Circadian effectiveness of solar and artificial radiation in dependence on age**
Helmut Piazena[1], Leonora Franke[1], David Sülflow[1], Katharina Stark[1], Dieter Kockott[2], Ralf Uebelhack[1] ([1]Berlin, [2]Hanau, GER)

Monday, September 3, Morning — Room 8W 2.1

9.45 **Photobiological techniques for environmental monitoring and control**
Chair: Francesco Ghetti (Pisa, ITA)

9.45 **IL231** **Recent developments in the application of PAM chlorophyll fluorometry**
Wolfgang Bilger (Kiel, GER)

10.15 **IL232** **Phytoremediation of metal polluted soils: a sunlight-driven environmentally friendly technique**
Franco Gambale, Monica Bregante (Genova, ITA)

10.45 **IL233** **Treatment of microbiologically polluted aquaculture waters by a novel photochemical technique of potentially low environmental impact**
Michela Magaraggia[1], Filippo Faccenda[2], Fabiola Paterno[2,1], Andrea Gandolfi[2], Giulio Jori[1] ([1]Padova, [2]Trento, ITA)

11.15 **IL234** **Automatic online bioassay system to monitor aquatic ecosystems using movement responses in unicellular microorganisms**
Donat-Peter Häder (Erlangen, GER)

11.30 **IL235** **Testing the presence of tributyl-Sn in marine water using microorganism behaviour**
Nicola Messina, Enrico De Gubernatis, Fernando Dini, Francesco Ghetti, Giovanni Checcucci (Pisa, ITA)

11.45 **Remembering Thameur Ben Amor, a pioneer in the field of photoinsecticides**
Giulio Jori (Padova, ITA)

11.50 **OC236** **Insecticidal effects of phloxine-B on *Bactrocera zonata* and its symbiotic bacteria**
Amira Abdou AlAdly (Cairo, EGY)

12.05 **OC237** **Photochemical transformation of dissolved organic matter in natural humic waters and humic isolate solutions**
Sarka Klementova, Petr Porcal, Jiri Kopacek, Dalibor Kriz (České Budějovice, CZE)

12.20 **P642** **Photoinactivation of bacteria in wastewater by porphyrins. Bacterial β-galactosidase activity and leucine-uptake as methods to monitor the process**
C.M B. Carvalho, A.T.P.C. Gomes, S.C.D. Fernandes, A.C.B. Prata, M.A. Almeida, M.A. Cunha, J.P.C. Tomé, M.A.F. Faustino, M.G.P.M.S. Neves, A.C. Tomé, J.A.S. Cavaleiro, Z. Lin, J.P. Rainho, J. Rocha (Aveiro, POR)

Monday, September 3, Afternoon — University Hall

16.00 **Drug delivery technologies for PDT**
Chair: Kristian Berg (Oslo, NOR)

16.00 **IL238** **Antimicrobial PDT: exploiting target cell function for selective photosensitizer delivery**
Tayyaba Hasan, Sarika Verma, Xiang Zheng, Ulysses Sallum, Humar Athar (Boston MA, USA)

16.30 **IL239** **Targeting proteolytic activity: new opportunities in fluorescence diagnosis and photodynamic therapy**
Norbert Lange (Geneva, SUI)

17.00 **IL240** **Recent improvements in the use of synthetic peptides for a selective photodynamic therapy**
Muriel Barberi-Heyob[1], Noémie Thomas[1], Loraine Tirand[1], Denise Bechet[1], Régis Vanderesse[2], François Guillemin[1], Céline Frochot[2] ([1]Vandoeuvre-les-Nancy, [2] Nancy, FRA)

17.30 **IL241** **Photochemical internalization: a technology for site-specific drug delivery**
Kristian Berg, Pål Kristian Selbo, Anette Bonsted, Anette Weyergang, Ole-Jacob Norum, Qian Peng, Anders Høgset (Oslo, NOR)

18.00 **OC242** **Development and characterisation of a protoporphyrin IX-peptide conjugate for use in the photodynamic therapy of cancer**
Clare Louise Conway (Leeds, GBR)

18.15 **OC243** **Synthesis of folate directed photodynamic therapy agents**
Kenneth W. Olsen, Godfred Boateng, Ping Hu (Chicago IL, USA)

18.30 **OC244** **Hypericin-loaded nanoparticles to improve photodetection of micrometastases in ovarian cancer**
Magali Zeisser-Labouèbe, Florence Delie, Robert Gurny, Norbert Lange (Geneva, SUI)

18.45 **OC245** **Photochemical internalisation can enhance the cytotoxicity of bleomycin and saporin in the A431 cell line**
Tzu-wen Wang, Stephen G. Bown, Alexander J. MacRobert (London, GBR)

Monday, September 3, Afternoon — Room 8W 1.1

16.00 **Photochemistry and photobiology of carotenoids**
Joint with the European Photochemistry Association
Chairs: Johan Lugtenburg (Leiden, NED), T. George Truscott (Keele, GBR)

16.00 **IL246** **Spheroidene in the *Rhodobacter sphaeroides* reaction centre. A surprise**
A.C. Wirtz[1], M.C. van Hemert[1], J. Lugtenburg[1], H.A. Frank[2], E.J.J. Groenen[1] ([1]Leiden, NED; [2]Storrs CT, USA)

16.30 **IL247** **Molecular basis of photoprotection in higher plants**
Bruno Robert (Gif-sur-Yvette, FRA)

17.00 **IL248** **Are dietary carotenoids anti-or pro-oxidants and are they beneficial or deleterious: a radical story**
T. George Truscott (Keele, GBR)

17.30 **IL249** **From photoprotection to photosensitization - Photoreactivity of the degradation products of carotenoids accumulating in the eye**
Malgorzata B. Rozanowska[1], Anna Pawlak[2], Bartosz Rozanowski[2] ([1]Cardiff, GBR; [2]Krakow, POL)

18.00 **IL250** **Oral suncare and photoprotection with carotenoids**
Regina Goralczyk (Basel, SUI)

18.30 **IL251** **Influence of molecular interactions on the natural colours of carotenoids**
George Britton (Liverpool, GBR)

Monday, September 3, Afternoon — Room 5W 2.3

16.00 **UV and oxidative stress**
Chair: Irene Kochevar (Boston MA, USA)

16.00 **IL252** **Signaling pathways in sunburn cell formation: from ROS to cell death**
An Van Laethem, Kris Nys, Marjan Garmyn, Patrizia Agostinis (Leuven, BEL)

16.30 **IL253** **Detecting Reactive Oxygen Species (ROS) in plants under UV stress**
Éva Hideg (Szeged, HUN)

17.00 **IL254** **Regulation of wound healing and UV response by peroxiredoxin 6**
Sabine Werner (Zürich, SUI)

17.30 **IL255** **UVA-induced reactive oxygen species in human keratinocytes**
Irene Kochevar (Boston MA, USA)

18.00 **OC256** **Melanocortin 1 receptor (MC1R) genotype influences erythemal sensitivity to PUVA**
Gillian Smith[1], Murray JV Wilkie[1], Yusuf Y Deeni[1], Peter M Farr[2], James Ferguson[1], C Roland Wolf[1], Sally H Ibbotson[1] ([1]Dundee, [2]Newcastle upon Tyne, GBR)

18.15 **P607** **Role of UV-induced oxidative stress in the regulation of the GTPase RhoB**
Bruno Canguilhem, Sarah Blaquière, Hélène Hernandez-Pigeon, Anne Pradines, Marie Charveron, Gilles Favre (Toulouse, FRA)

18.30 **P614** **Singlet oxygen-mediated tryptophan oxidation: characterization of potential markers of protein oxidation**
Michelle Gracanin, David I. Pattison, Michael J. Davies (Sydney, AUS)

18.45 **P626** **Spectrophotometric study of antioxidant and iron binding capacity of selected flavonoid compounds**
George Zonios, Aikaterini Dimou, Dimitrios Galaris (Ioannina, GRE)

Monday, September 3, Afternoon — Room 8W 2.1

Photoorientation in plants and microorganisms

Chair: Francesco Lenci (Pisa, ITA)

16.00 **IL257** **Diurnal and circadian expression of genes encoding opsin-like photoreceptor proteins in *Hydra* (Cnidaria, Hydrozoa)**
Silvia Santillo, Pierangelo Orlando, Luciano De Petrocellis, Carlo Musio (Napoli, ITA)

16.30 **IL258** **Confocal microscopy in ciliate protozoa**
Giuliano Colombetti[1], Paolo Bianchini[2], Ranieri Bizzarri[1], Giovanni Checcucci[1], Alberto Diaspro[2], Sabina Lucia[1], Mattia Pesce[2], Paola Ramoino[2], Cesare Usai[2], Giuseppe Vicidomini[2] ([1]Pisa, [2]Genova, ITA)

Tuesday, September 4, Morning — University Hall

08.30 **PL301** **Photobiology Update**
From photochemistry and photobiology to photobiotechnology of plants
Pill-Soon Song (Jeju, KOR) *introduced by Francesco Lenci (Pisa, ITA)*

09.15 **Coffee Break**

9.45 **Biological consequences of UV damage**
Chairs: Alain Sarasin (Villejuif, FRA), Carlos F.M. Menck (São Paulo, BRA)

9.45 **IL302** **A novel role for c-Fos in the defence against ultraviolet light**
Bernd Kaina (Mainz, GER)

10.15 **IL303** **Molecular responses of normal human keratinocytes in culture and in reconstructed skin to genotoxicity induced by solar UV: towards a genomic protection factor *in vitro***
Laurent Marrot, Jean-Philippe Belaidi, Christophe Jones, Jean-Roch Meunier (Aulnay-sous-Bois, FRA)

10.45 **IL304** **Viral vectors to explore the consequences of UV-induced DNA damage**
Carlos F.M. Menck (São Paulo, BRA)

11.15 **IL305** **Molecular mechanisms of UV-induced mutagenesis in human cells**
Alain Sarasin[1], Anne Stary[1], Quentin Gueranger[2], Claude-Agnès Reynaud[2], Jean-Claude Weill[2] ([1]Villejuif, [2]Paris, FRA)

11.45 **OC306** **The UV-B damage response in *Daphnia* spp.: diverse repair strategies at 10 and 20°C**
Sandra J Connelly[1], Craig E Williamson[1], David L Mitchell[2] ([1]Oxford OH, [2]Smithville TX, USA)

12.00 **OC307** **Association of the extracellular chaperone clusterin with altered elastic fibers *in vivo* and *in vitro***
Elke Janig[1], Martin Halsbeck[2], Johannes Buchner[2], Ariane Aigelsreiter[1], Helmut Denk[1], Kurt Zatloukal[1], Peter Wolf[1] ([1]Graz, AUT; [2]Garching - Munich, GER)

12.15 **OC308** **After UVB irradiation, p21 expression is regulated by p53 through DNA-protein contact and protein-protein interactions**
Nathalie Bastien[1], Walid Dridi[1], Isabelle Paradis[1], Mylene Blais[1], Raouf Fetni[2], Claude Asselin[1], Regen Drouin[1] ([1]Sherbrooke, [2]Montreal, CAN)

Tuesday, September 4, Morning — Room 8W 1.1

Biophysics of photosensitization

Chair: Benjamin Ehrenberg (Ramat Gan, ISR)

9.45 **IL309** **The photosensitized production and optical detection of singlet oxygen with sub-cellular resolution in single cells**
Peter R. Ogilby (Aarhus, DEN)

10.15 **IL310** **Singlet oxygen production by proteins from the green fluorescent protein family**
Santi Nonell[1], Ana Jiménez-Banzo[1], Johan Hofkens[2], Cristina Flors[2] ([1]Barcelona, ESP; [2]Leuven, BEL)

10.45 **IL311** **Depolarization of cross-membrane electric potential in photosensitization**
Benjamin Ehrenberg, Shoshana Bernstein (Ramat Gan, ISR)

11.15 **IL312** **Analysis of ALA-induced PPIX across multiple cell lines: variance and dependence upon physical parameters**
Summer L. Gibbs, Scott C. Davis, Julie A. O'Hara, Brian W. Pogue (Hanover NH, USA)

11.45 **OC313** **Time resolved luminescence and singlet oxygen formation under illumination of hypericin in complex with low density lipoproteins**
Peter Gbur[1], Daniel Jancura[1], Roman Dedic[2], Dusan Chorvat jr.[3], Jan Hala[2], Pavol Miskovsky[1,3] ([1]Kosice, [3]Bratislava, SVK; [2]Prague, CZE)

12.00 **OC314** **Role of plasma LDL and tumoral microenvironment in tetrapyrrole-photosensitizers cellular uptake: a physico-chemical approach**
Stéphanie Bonneau, Halina Mojzisova, Christine Vever-Bizet, Daniel Brault (Paris, FRA)

12.15 **OC315** **Reflectance of skin in the UV region**
Johan Moan[1], Asta Juzeniene[1], Kristian P. Nielsen[2] ([1]Oslo, [2]Bergen, NOR)

Tuesday, September 4, Morning — Room 5W 2.3

9.45

A reconstruction of the past UV climatology over Europe for photobiological studies
Joint with the EU Programme COST 726

Chair: Gaetano Zipoli (Firenze, ITA)

9.45 **IL316** **COST 726: Long term changes and climatology of UV radiation over Europe**
Alois W. Schmalwieser (Vienna, AUT)

10.15 **IL317** **Quality of UV measurements**
Mario Blumthaler (Innsbruck, AUT)

10.45 **IL318** **Modeling UV radiation in the past: achievements and limitations**
Peter Koepke[1], Jean Verdebout[2] ([1]Munich, GER; [2]Ispra, ITA)

11.15 **IL319** **The role of action spectra in determining the biologically effective UV radiation**
Gaetano Zipoli, Daniele Grifoni (Firenze, ITA)

11.45 **OC320** **Exploring the details of UV irradiances, human exposure and dosimetry**
Richard Kift, Liam McNulty, Lucy Bunhill, Marie Durkin, Donald Allan, Jacqueline Berry, Lesley E. Rhodes, Ann R. Webb (Manchester, GBR)

12.00 **OC321** **Reconstructed long-term erythemal irradiance over Europe from measurements of solar irradiance and total ozone**
Andreas Kazantzidis[1], Alkiviadis Bais[1], Peter Den Outer[2], Harry Slaper[2], Tapani Koskela[3], Uwe Feister[4], M. Woldt[4] ([1]Thessaloniki, GRE; [2]Bilthoven, NED; [3]Jokioinen, FIN; [4]Lindenberg, GER)

12.15 **OC322** **Photoprotection and skin cancer prevention in the Czech caucasion population**
Michal Janouch, Karel Ettler (Hradec Kralove, CZE)

12.30 **OC323** **UVBE maps for Poland – preliminary results for selected action spectra**
Zenobia Litynska[1], Aleksander Curylo[1], Bozena Lapeta[2], Julita Biszczuk[1], Janusz Krzyscin[3], Barbara Bogdanska[3], Jakub Walawender[2] ([1]Legionowo, [2]Krakow, [3]Warsaw, POL)

Tuesday, September 4, Morning — Room 8W 2.1

9.45

Photodermatoses
In memoriam of Ian Magnus (1920-2006)
Joint with the British Photodermatology Group

Chairs: Neil Gibbs (Manchester, GBR), Antony Young (London, GBR)

9.45 **Intoductory remarks**
Neil Gibbs[1], Antony Young[2] ([1]Manchester, [2]London, GBR)

9.50 **A century of photodermatology**
Herbert Hönigsmann (Vienna, AUT)

10.20 **IL324** **Skin phototesting**
Brian Diffey (Newcastle, GBR)

10.50 **IL325** **The porphyrias**
Gillian M Murphy (Dublin, IRL)

11.20 **IL326** **The immunological photodermatoses**
John L M Hawk (London, GBR)

11.50 **OC327** **Photosensitive psoriasis: clinical characteristics and potential role of memory effector T lymphocytes in the pathogenesis**
Kirsty J Rutter, Lindsey F Cotterell, Margaret M Brownrigg, Thomas Brenn, Christopher EM Griffiths, Rachel EB Watson, Lesley E Rhodes (Manchester, GBR)

12.05 **OC328** **High levels of anxiety and depression related to their condition reported in a hospital based PLE population**
Tsui C. Ling, H.L. Richards, M. Brownrigg, R.C.C. Brooke, K. Huber, N.K. Gibbs, L.E. Rhodes (Manchester, GBR)

12.20 **OC329** **The effect of capsaicin-induced neuropeptide depletion on the induction of polymorphous light eruption by solar simulated light**
Franz J Legat, Agnes Bretterklieber, Manuela Haar, Goran Sepic, Angelika Hofer, Alexandra Wackernagel, Franz Quehenberger, Helmut Kerl, Peter Wolf (Graz, AUT)

Tuesday, September 4, Afternoon — University Hall

14.15 **Photochemistry and phototoxicity of drugs**
Chair: Miguel A. Miranda (Valencia, ESP)

14.15 **IL330** ***In vitro*** **phototoxicity testing: a task involving multiple endpoints**
Laurent Marrot, Jean-Philippe Belaidi, Christophe Jones, Anita Labarussiat, Philippe Perez, Jean-Roch Meunier (Aulnay-sous-Bois, FRA)

14.45 **IL331** **Dermatological overview of phototoxic reactions: a mechanistic approach**
Paulo Leal Filipe[1], Patrice Morlière[2], René Santus[3] ([1]Lisbon, POR; [2]Amiens, [3]Paris, FRA)

15.15 **IL332** **Effects of UVB light on antiinflammatory corticosteroids in different experimental models**
Sergio Caffieri, Giorgia Miolo (Padova, ITA)

15.45 **IL333** **Phototoxicity of tricyclic antidepressants**
Carmelo Garcia[1], Rolando Oyola [1], Luis E. Piñero [1], Rafael Arce[2] ([1]Humacao, [2]Rio Piedras, PUR)

16.15 **OC334** **Site-selected photoelectron transfer and covalent binding in Nalidixic Acid:HSA complexes**
Sandra Monti, Ilse Manet, Francesco Manoli, Massimo Capobianco (Bologna, ITA)

16.45 **OC335** **Primary photoprocesses in some photosensitive drugs studied using fast reaction kinetic techniques**
Suppiah Navaratnam (Salford, Warrington, GBR)

17.00 **OC336** **Photodehalogenation of fluoroquinolones**
M. Consuelo Cuquerella, Francisco Boscá, Miguel A. Miranda (Valencia, ESP)

Tuesday, September 4, Afternoon — Room 8W 1.1

14.15 **PDT oral communications**
Chair: Jacques Piette (Lièges, BEL)

14.15 **OC 337** **Mechanisms of the selective photodynamic efficiency of the hexyl-aminolevulinate (h-ALA)-protoporphyrine IX (PpIX) in the treatment of the bladder cancer**
Berrahmoune Saoussen[1], EL Khatib Sami[1], Brie David[1], Fotinos Nicolas[2], Guedenet Jean-Claude[1], Bezdetnaya Lina[1], Guillemin François[1], D'Hallewin Marie-Ange[1] ([1]Vandœuvre-lès-Nancy, FRA; [2]Genève, SUI)

14.30 **OC 338** **Effects of hexyl 5-aminolevulinate and light in a rat bladder cancer model**
Odrun Gederaas, Lise Lyngsnes Randeberg, Eivind La Puebla Larsen, Astrid Hjelde, Carl-Jørgen Arum, Anders Brunsvik, Chun-Mei Zhao, Duan Chen, Lars Ottar Svaasand, Hans E. Krokan (Trondheim, NOR)

14.45 **OC 339** **MAL-PDT of squamous cell carcinoma**
Piergiacomo Calzavara- Pinton, Raffaella Sala, Marina Venturini, Giovanni Parrinello, Cristina Zane (Brescia, ITA)

15.00 **OC 340** **Protoporphyrin IX production and photodynamic effects after application 5-aminolevulinic acid or its heptyl ester**
Asta Juzeniene[1], Xiao Puzhuoma[2], Johan Moan[1] ([1]Oslo, NOR; [2]Lhasa Tibet, CHN)

15.15 **OC 341** **Using iron chelating agents to enhance PPIX-induced photodynamic therapy**
Alison Curnow, Andrew Pye, Sandra Campbell (Truro, GBR)

15.30 **OC 342** **Photodynamic-mediated proteasome paralysis reinforced by non toxic drug may force tumour cells to apoptosis**
Giuseppe Palumbo, Angela Chiaviello (Napoli, ITA)

15.45 **OC343** **ALA-PDT and wound healing**
Stefania Motta, Antonella Ferrario , Marcello Monti (Milano, ITA)

16.00 **OC344** **Plea for the identification and development of new sensitizers**
Thierry Patrice, David Olivier, Samuel Douillard (Nantes, France)

16.15 **OC345** **Photochemical internalization (PCI) of the recombinant melanoma-targeting immunotoxin scFvMEL/rGel causes rapid tumor regression *in vivo***
Pål K. Selbo[1], Lawrence H. Cheung[2], Wendy Zhang[2], Michael G. Rosenblum[2], Kristian Berg[1] ([1]Oslo, NOR; [2]Houston TX, USA)

16.30 **OC346** **An *in vitro* investigation of the mechanisms of δ-aminolaevulinic-acid (ALA)-photodynamic therapy (PDT)**
Yuktee Dogra, Alison Curnow, Paul Winyard (Exeter, GBR)

16.45 **OC347** **The effect of photodynamic therapy with verteporfin on epidermal growth factor receptor signaling**
Thomas Stepinac, Tayyaba Hasan (Boston MA, USA)

17.00 **OC348** **Factors affecting substrate specificity of photosensitizers for the multidrug-resistance pump ABCG2 found on some cancers**
Janet Morgan, Ravindra K Pandey, Allan R Oseroff (Buffalo NY, USA)

Tuesday, September 4, Afternoon — Room 5W 2.3

14.15 **Photoprotection**
Joint with the European Society for Photodermatology
Chairs: Christophe Bédane (Limoges, FRA), Piergiacomo Calzavara-Pinton (Brescia, ITA)

14.15 **IL349** **Endogenous photoprotection by melanin**
Bernhard Ortel (Chicago IL, USA)

14.45 **IL350** **UVA and UVB effects on melanocytic nevi**
Piergiacomo Calzavara-Pinton, Marina Venturini, Ausilia Manganoni (Brescia, ITA)

15.15 **IL352** **Oral photoprotection**
Rik Roelandts (Leuven, Belgium)

15.45 **OC353** **Analysis of UVA and UVB irradiations on melanocytes: evaluation of the protective capacity of a sunscreen**
Hélène Hernandez-Pigeon, Marie-José Haure, Sawsane Bakioui, Françoise Belaubre, Marie Charveron (Toulouse, FRA)

16.00 **OC354** **UV-Filter combinations under UV-A exposure: concomitant quantification of over-all spectral stability and molecular integrity**
Elisabetta Damiani, Lucedio Greci (Ancona, ITA)

16.15 **OC355** **Impact of dietary lycopene on molecular markers of photoageing in human skin**
Isabel Rodriguez-Blanco, Muneeza Rizwan, Lesley Elizabeth Rhodes, Rachel Watson (Manchester, GBR)

16.30 **OC356** **The role of sunscreens in the prevention of skin cancer: assessing the correlation between protection against erythema and DNA photodamage**
Suranahi K Buglass, Antony R Young (London, GBR)

16.45 **OC357** **UV and visible free radical action spectrum for human skin and its biological relevance**
Leonhard Zastrow[1], Louis Ferrero[1], Dieter Kockott[2], Frank Klein[3], Norbert Groth[3], Jürgen Lademann[2] ([1]Monaco, MON; [2]Hanau-Stenheim, [3]Berlin, GER)

17.00 **OC358** **Analysis of the properties of different substrates useful for *in vitro* sunscreen tests**
D. Garoli, M.G. Pelizzo, P. Nicolosi, A. Peserico, M. Alaibac (Padova, ITA)

Tuesday, September 4, Afternoon — Room 8W 2.1

14.15 **UV and global climate change: effects in aquatic and terrestrial ecosystems**
Joint with the America Society for Photobiology
Chairs: Donat-Peter Häder (Erlangen, GER), Patrick Neale (Edgewater MD, USA)

14.15 **IL359** **Active and passive mitigating strategies of aquatic primary producers against excessive solar radiation**
Donat-Peter Häder (Erlangen, GER)

14.40 **IL360** **Sensitivity to solar radiation of summer phytoplankton assemblages from mid-latitudes of Patagonia**
E. Walter Helbling[1], M. Alejandra Marcoval[1], Paul P.Janknegt[2], Marco de Graaff[2], Ronald J. W. Visser[2], Anita G. J. Buma[2], Virginia E. Villafañe[1] ([1]Playa Unión-Rawson, ARG; [2]Haren, NED)

15.05 **IL361** **Ultraviolet radiation tolerance and photoenzymatic repair across aquatic taxa from bacteria to fish: implications for climate change**
Craig E Williamson (Oxford OH, USA)

15.30 **IL362** **Ultraviolet radiation effects on phytoplankton productivity in a changing global climate**
Patrick J. Neale[1], Cristina Sobrino[2] ([1]Edgewater MD, USA; [2]Vigo, ESP)

15.55 **IL363** **Plant responses to UV radiation: from signalling pathways to sustainable production**
Nigel D. Paul[1], Gareth Jenkins[2], Jason J. Wargent[1] ([1]Lancaster, [2]Glasgow, GBR)

16.20 **OC364** **Fitting action spectra: ambient levels of UV-A and UV-B radiation affect phenolic compounds in birch leaves**
Titta Katariina Kotilainen[1], Tuulia Venäläinen[1], Riitta Tegelberg[2], Riitta Julkunen-Tiitto[3], Pedro Jose Aphalo[2] ([1]Jyväskylä, [2]Helsinki, [3]Joensuu, FIN)

16.33 **OC365** **How the DNA composition and the physiological state of marine bacteria may modify their DNA damages and their sensitivity to UV radiation**
Sabine Matallana Surget[1,2], Fabien Joux[1], Jarah Meador[1,3], Thierry Douki[3], Rick Cavicchioli[2] ([1]Banyuls-sur-mer, [3]Grenoble, FRA; [2]Sydney, AUS)

16.46 **OC366** **The effects of UV radiation on multitrophic responses and their implications for sustainable agroecosystem management**
Jason J. Wargent, Nigel D. Paul (Lancaster, GBR)

16.59 **OC367** **Singlet oxygen affects activity, cells size and species composition in a dystrophic humic acid lake**
Stefanie Glaeser[1], Bork Berghoff[1], Hans-Peter Grossart[2], Jens Glaeser[1] ([1]Giessen, [2]Stechlin, GER)

Tuesday, September 4, Afternoon — University Hall

17.15 **ESP General Assembly**

Tuesday, September 4, Evening — Assembly Rooms

19.30 **Banquet – ESP Medal Awards**

Wednesday, September 5, Morning — University Hall

08.30 **PL401** **Photobiology Update**
Impacts of UV-B radiation on biotic and abiotic systems in relation to global climate change
Janet F. Bornman (Hamilton, NZL) *introduced by Francesco Ghetti (Pisa, ITA)*

09.15 **Coffee Break**

9.45 **Molecular and cellular effects of UV-A**
Chair: Rex M. Tyrrell (Bath, GBR)

9.45 **The role of mitochondria and lipid rafts in UVA-radiation-induced signaling and premature skin aging**
Jean Krutmann (Düsseldorf, GER)

10.15 **IL403** **Mechanisms of UVA- and sunlight-induced mutations**
Gerd P. Pfeifer, Sang-in Kim, Ahmad Besaratinia (Duarte CA, USA)

10.45 **IL404** **UVA-induced response in eucaryotic cells**
Evelyne Sage[1], Violetta Smirnova[1], Anne Reynaud-Angelin[1], Pierre-Marie Girard[1], Stefania Francesconi[1], Delphine Dardalhon[1], Ludovic Tessier[1], Stanislav Kozmin[1], Thierry Douki[2] ([1]Orsay, [2]Grenoble, FRA)

11.15 **IL405** **Genotoxic effects of UVA radiation on human cells and skin: photosensitized reactions**
Jean Cadet, Stephane Mouret, Jean-Luc Ravanat, Thierry Douki (Grenoble, FRA)

11.45 **IL406** **UVA stress to human skin cells and the maintenance of heme and iron homeostasis**
Rex Michael Tyrrell, Chintan Raval, Gavin Edwards, Julia Zhong (Bath, GBR)

12.15 **OC407** **Tryptophan-derived UV-filter compounds covalently bound to lens proteins are photosensitizers of oxidative damage**
Jasminka Mizdrak, Joanne F. Jamie, Peter G. Hains, Roger J.W. Truscott, Michael J. Davies (Sydney, AUS)

Wednesday, September 5, Morning — Room 8W 1.1

9.45 **New approaches to clinical phototherapy**
Chair: Peter Wolf (Graz, AUT)

9.45 **IL408** **311 nm UVB *vs*. PUVA: is 311 nm always best?**
John L M Hawk (London, GBR)

10.10 **IL409** **Topical PUVA**
Herbert Hönigsmann (Vienna, AUT)

10.35 **IL410** **UVA-1: has it kept its promise?**
Adrian Tanew (Vienna, AUT)

11.00 **IL411** **Extracorporeal photoimmunotherapy: an update**
Robert Knobler (Vienna, AUT)

11.25 **IL412** **Molecular mechanisms of photochemotherapy: significance of PAF and 5-HT**
Peter Wolf (Graz, AUT)

11.50 **IL413** **Molecular risk assessment of rhinophototherapy for the treatment of seasonal allergies**
David L Mitchell[1], Andrea Koreck[2], Edina Garaczi[2], Zsolt Bella[2], Lajos Kemeny[2] ([1]Smithville TX, USA; [2]Szeged, HUN)

12.15 **OC414** **Erythema effectiveness of long-wave UV radiation - LASERS versus conventional radiation**
Helmut Piazena, Hans Meffert (Berlin, GER)

12.27 **OC415** **Concomitant treatment of chronic plaque psoriasis with initial pulsed dye laser and narrow-band UV-B therapy**
Angelika Hofer, Daisy Kopera, Franz Legat, Helmut Kerl, Peter Wolf (Graz, AUT)

12.39 **OC416** **Trafficking of 8-MOP treated leucocytes after photopheresis in humans**
Ulrike Just[1], Elke Dimou[1], Robert Knobler[1], Gabriele Klosner[1], Erika Ivancic[1], Hildegard Greinix[1], Alexander Becherer[2], Franz Trautinger[3] ([1]Vienna, [2]Feldkirch, [3]St Pölten, AUT)

12.51 **OC417** **Synergies of VEGF inhibition and photodynamic therapy in the treatment of age-related macular degeneration**
Maria Fernanda Zuluaga[1], Carolina Mailhos[2], Gregory Robinson[2], David T. Shima[2], Robert Gurny[1], Norbert Lange[1] ([1]Geneva, SUI; [2]Lexington MA, USA)

Wednesday, September 5, Morning — Room 8W 2.1

9.45 **PDT, light and the immune system**

Chair: Gianfranco Canti (Milano, ITA)

9.45 **IL418** **Photodynamic therapy stimulates anti-tumor immunity in murine models**
Michael R Hamblin, Pawel Mroz, Ana P Castano (Boston MA, USA)

10.15 **IL419** **Local phototherapy and immunotherapy for treatment of metastatic tumors**
Wei R. Chen (Edmond OK, USA)

10.45 **IL420** **Photodynamic therapy-generated vaccine: therapeutic and mechanistic insights**
Mladen Korbelik (Vancouver, CAN)

11.15 **IL421** **Innate cells play a critical role in enhancement of anti-tumor immunity by PDT**
Sandra O. Gollnick (Buffalo NY, USA)

11.45 **OC422** **Targeting inflamed synovium with protease-sensitive photodynamic agents**
Doris Gabriel[1], Norbert Lange[1], Marino Andres Campo[1], Veronique Chobaz-Péclat[2], Robert Gurny[1], Alexander So[2], Nathalie Busso[2] ([1]Geneva, [2]Lausanne, SUI)

12.15 **OC423** **Photodynamic therapy induces immunity against a beta-galactosidase expressing tumor**
Pawel A Mroz, Michael R Hamblin (Boston MA, USA)

Wednesday, September 5, Afternoon — University Hall

16.00 **PDT to treat microbial infections**
***In memoriam* of John D. Spikes (1918-2006)**
Chair: Giulio Jori (Padova, ITA)

16.00 **IL424** **Advances in antimicrobial photodynamic therapy**
Michael R Hamblin[1], George P Tegos[1], Aguinaldo S Garcez[2] ([1]Boston MA, USA; [2]São Paulo, BRA)

16.30 **IL425** **Photosensitisers for antimicrobial PDT: physico-chemical and photobiological properties**
Giulio Jori (Padova, ITA)

17.00 **IL426** **Potential of PDT for control of parasitic infection**
Ross William Boyle[1], Timothy Paget[2], Carrie-Anne Bristow[1] ([1]Kingston-upon-Hull, [2]Chatham, GBR)

17.30 **IL427** **Light-activated antimicrobial agents for the treatment of periodontitis - a clinical evaluation**
Michael Wilson (London, GBR)

18.00 **IL428** **A double-blinded, randomised, placebo controlled clinical trial to determine whether PDT using the phenothiazinium salt, PPA 904 can reduce bacterial load in chronic leg ulcers and chronic diabetic foot ulcers**
S.B. Brown[1], C. O'Grady[1], K. Mellish[1], J. Griffiths[1], H. Moseley[2], M. Rizwan[3], L.E. Rhodes[3], S.M. Morley[2] ([1]Leeds, [2]Dundee, [3]Manchester, GBR)

18.30 **OC429** **Enzyme-activated photosensitizers for use in antimicrobial photodynamic therapy**
Sarika Verma, Ulysses Sallum, Humra Athar, Gregory Watt, Tayyaba Hasan (Boston MA, USA)

18.45 **OC430** **The green line of frequency doubled Neodymium:YAG Laser for killing the sensitized pathogenic bacteria in comparison with the red light of He:Ne Laser**
Siham A Kandela, Alic K Melconian (Baghdad, IRQ)

Wednesday, September 5, Afternoon — Room 8W 1.1

16.00 **Experimental photocarcinogenesis**
Chairs: Frank R de Gruijl (Leiden, NED), Honnavara N. Ananthaswamy (Houston TX, USA)

16.00 **IL431** **Forced proliferation of epidermal stem and progenitor cells with accumulated UV-induced DNA damage gives rise the foci of cells overexpressing wild type p53**
Joanne GW Nijhof, Leon HF Mullenders, Frank R de Gruijl (Leiden, NED)

16.30 **IL432** **Protein Kinase C epsilon interacts with Stat3 and regulates its activation to sensitize skin to ultraviolet radiation-induced development of squamous cell carcinomas**
Ajit K Verma (Madison WI, USA)

17.00 **IL433** **Blocking serotonin and/or platelet activating factor receptor binding interferes with photocarcinogenesis**
Stephen E. Ullrich (Houston TX, USA)

17.30 **IL434** **Topical tacrolimus in combination with simulated solar radiation does not enhance photocarcinogenesis in hairless mice**
Catharina M. Lerche[1], Peter A. Philipsen[1], Thomas Poulsen[2], Hans Christian Wulf[1] ([1]Copenhagen, [2]Soenderborg, DEN)

18.00 **OC435** **UV-induced carcinogenesis in a p53 mutant mouse model: increased sensitivity to skin damage by a point mutation at codon 172**
Cara L. Benjamin, Chengming Zhu, Honnavara N. Ananthaswamy (Houston TX, USA)

18.15 **OC436** **Deregulation of the cell death response to UVB and cisplatin in squamous cell carcinoma cell lines**
Sofie Claerhout[1], An Van Laethem[1], Lien Verschooten[1], Charlotte Proby[2], Patrizia Agostinis[1], Marjan Garmyn[1] ([1]Leuven, BEL; [2]London, GBR)

18.30 **OC437** **Changes in epigenetic regulation of chromatin in human epidermis and primary human skin cells after UV-irradiation *in vivo* and *in vitro***
Ruediger Greinert, Kristina Behrend, Beate Volkmer (Buxtehude, GER)

Wednesday, September 5, Afternoon — Room 8W 2.1

16.00 **Mechanistic and applied approaches to plant response in a changing climate**
Chair: Janet F. Bornman (Hamilton, NZL)

16.00 **IL438** **Exploiting plant UV responses for sustainable crop production in a changing climate**
Nigel D. Paul, Jason M. Moore, Jason J. Wargent (Lancaster, GBR)

16.30 **IL439** **Interaction of UV-B radiation and low temperature: damage and acclimation**
Wolfgang Bilger, Karena Hoffmann-Wülfing, Matthias Schultze (Kiel, GER)

17.00 **IL440** **Flavonoids and iridoids in plants: their role and responses to environmental change**
Ken G Ryan[1], Rilka Taskova[1], Allan Burne[1], Lisa Bryant[1], Rod Seppelt[2], Phil Garnock-Jones[1] ([1]Wellington, NZL; [2]Hobart, AUS)

17.30 **OC441** **Plant response action spectra in the spot-light**
Emma McLoughlin[1], Kevin Newsham[2], John Moncrieff[1], Andrew McLeod[1] ([1]Edinburgh, [2]Cambridge, GBR)

17.45 **OC442** **Effect of UV-B light and temperature on primary photosynthetic processes in two lichen species from contrasting habitats**
Hana Vrablikova[1], Per Larsson[2], Kristyna Vecerova[1], Knut-Asbjorn Solhaug[2], Yngvar Gauslaa[2] ([1]Brno, CZE; [2]Aas, NOR)

Thursday, September 6, Morning — University Hall

08.30 **PL501** **Photobiology Update**
DNA repair from past to future
Leon H.F. Mullenders (Leiden, NED) *introduced by Jean Cadet (Grenoble, FRA)*

09.15 **Coffee Break**

9.45 **Photodiagnosis and optical techniques for medical diagnosis**
Chair: Georges A. Wagnieres (Lausanne, SUI)

9.45 **IL502** **Breast characterization and lesion detection by means of optical spectroscopy and imaging**
Paola Taroni, Daniela Comelli, Antonio Pifferi, Lorenzo Spinelli, Alessandro Torricelli, Gianmaria Danesini, Rinaldo Cubeddu (Milano, ITA)

10.15 **IL503** **Optical signatures for ovarian cancer screening**
Urs Utzinger[1], Molly Brewer[2] ([1]Tucson AZ, [2]Farmington CT, USA)

10.45 **IL504** **Differential Pathlength Spectroscopy for medical diagnosis**
H.J.C.M. Sterenborg, B.Kruijt, S.Kaskakova, H.S. de Bruijn, D.J.Robinson, A.Amelink (Rotterdam, NED)

11.15 **OC505** ***In vivo* imaging of the bronchial and alveolar walls microstructure using fibered confocal autofluorescence microscopy**
Genevieve Bourg-Heckly[1], Luc Thiberville[2], Sophie Moreno-Swirc[2], Christine Vever-Bizet[1] ([1]Paris, [2]Rouen, FRA)

11.30 **OC506** **Development of microcystoscopy for the characterization of lesions detected by fluorescence imaging with Hexvix® in the human bladder**
Blaise Lovisa, Patrice Jichlinski, Daniela Aymon, Hubert van den Bergh, Georges A. Wagnieres (Lausanne, SUI)

11.45 **OC507** ***In vivo* optical properties of melanin and non-invasive detection of melanoma**
George Zonios[1], Aikaterini Dimou[1], Ioannis Bassukas[1], Dimitrios Galaris[1], Efthimios Kaxiras[2] ([1]Ioannina, GRE; [2]Cambridge MA, USA)

12.00 **OC508** **Correlation between PPIX fluorescence and both tissular effects and pain induced by PDT on normal skin using fluorescence imaging**
Jérôme Barge, Thomas Glanzmann, Hubert van den Bergh, Georges Wagnières (Lausanne, SUI)

12.15 **OC509** **Fluorescence dynamics studies of a PDT photosensitizer**
Muhammad Atif (Islamabad, PAK)

12.30 **OC510** **Synthesis and photophysical study of fluorescent bile acid derivatives suitable for anion transport measurements on hepatocytes**
Maria Luisa Marin, Jana Rohacova, Miguel Angel Miranda, Maria José Gómez-Lechón, José Vicente Castell (Valencia, ESP)

Thursday, September 6, Morning — Room 8W 1.1

9.45

Recent advances in the characterization of photooxidative damage at the biomolecular level

Chairs: Albert Girotti (Milwaukee WI, USA), Colin Chignell (Research Triangle Park NC, USA)

9.45 **IL511** **Oxygen 18-labeled singlet molecular oxygen as a valuable tool for studying DNA damage**
Paolo Di Mascio[1], Glaucia R. Martinez[2], Marisa H.G. Medeiros[1], Didier Gasparutto[3], Jean-Luc Ravanat[3], Jean Cadet[3] ([1]São Paulo SP, [2]Curitiba, BRA; [3]Grenoble, FRA)

10.15 **IL512** **Mechanisms of cellular damage induced by peptide- and protein-peroxides generated by photooxidation**
Michael J. Davies (Sydney, AUS)

10.45 **IL513** **UVA sensitivity in Smith-Lemli-Opitz Syndrome: possible involvement of cholesta-5,7,9(11)-trien-3β-ol**
Colin F. Chignell, Robert H. Sik, Piotr J. Bilski, Yu-Ying He, Barbara M. Kukielczak (Research Triangle Park NC, USA)

11.15 **IL514** **Role of cardiolipin peroxidation in apoptotic cell death induced by mitochondrial photooxidative damage**
Albert W Girotti[1], Witek Korytowski[1,2], Tamas Kriska[1] ([1]Milwaukee WI, USA; [2]Krakow, POL)

11.45 **OC515** **Adaptive responses to singlet oxygen generated stress in *Rhodobacter***
Jens Glaeser[1], Monica Zobawa[2], Friedrich Lottspeich[2], Gabriele Klug[1] ([1]Giessen, [2]Martinsried, GER)

Thursday, September 6, Morning — Room 8W 2.1

9.45 **Blue light regulation in plants and microorganisms**
Chair: Aba Losi (Parma, ITA)

9.45 **IL516** **Photoreceptor genes in the fungus *Phycomyces blakesleeanus***
Catalina Sanz[1], Julio Rodriguez-Romero[2], Alex Idnurm[3], Victor G. Tagua[2], Luis M. Corrochano[2], Joseph Heitman[3], Arturo P. Eslava[1] ([1] Salamanca, [2] Sevilla, ESP; [3]Durham NC, USA)

10.15 **IL517** **Structural and functional studies of the *Bacillus subtilis* blue-light sensor YtvA and related proteins**
Aba Losi (Parma, ITA)

10.45 **IL518** **Spectroscopic and structural characterization of a plant member of the cryptochrome DASH subfamily**
Richard Pokorny, Julia Moldt, Tobias Klar, Lars-Oliver Essen, Alfred Batschauer (Marburg, GER)

11.15 **IL519** **Light regulates virulence in *Brucella abortus* by a LOV- Domain Histidine Kinase protein**
Gastón Paris[1], Diego J. Comerci[1], Trevor E. Swartz[2], Roberto Bogomolni[2], Rodolfo A. Ugalde[1], Fernando A. Goldbaum[1] ([1]Buenos Aires, ARG; [2]Santa Cruz CA, USA)

11.45 **OC520** **Ultrafast studies on novel blue-light photoreceptors**
Cosimo Bonetti[1], Tilo Mathes[2], Ivo H. M. van Stokkum[1], Marie-Louise Groot[1], Rienk van Grondelle[1], Peter Hegemann[2], John T.M. Kennis[1] ([1]Amsterdam, NED; [2]Berlin, GER)

12.00 **OC521** **Radical formation and autophosphorylation in the light-sensitive domain of the plant cryptochrome from *Chlamydomonas***
Dominik Immeln[1,2], Ramona Schlesinger[2], Joachim Heberle[1], Tilman Kottke[1,2] ([1]Bielefeld, [2]Jülich, GER)

Monday, September 3, 14.15 – 15.45 **Sports Hall at Founders Complex**

Poster session I

P601 **UVB-induced DNA damage: high proportion of mutagenic photoproducts in GC-rich genomes**
Sabine Matallana Surget[1], Jarah Meador[1,2], Fabien Joux[1], Thierry Douki[2] ([1]Banyuls-sur-mer, [2]Grenoble, FRA)

P602 **Photoactivated hypericin-mediated DNA damage in HaCaT keratinocytes can be attenuated by vitamin E**
Brian Johnston, Nicola J Traynor, Harry Moseley, James Ferguson, Julie A Woods (Dundee, GBR)

P603 **The crystal and molecular structure of the d(TpA) thymine-adenine photoadduct**
R. Jeremy H. Davies[1], John F. Malone[1], Yu Gan[2], Christine J. Cardin[2], Michael P. H. Lee[3], Stephen Neidle[3] ([1]Belfast, [2]Reading, [3]London, GBR)

P604 **Radiation-induced clustered DNA damage**
Noureddine Belmadoui, Peggy Regulus, Jean-Luc Ravanat, Didier Gasparutto, Jean Cadet (Grenoble, FRA)

P605 **The GTPase RhoB, a UVB-responsive protein, regulates p53-independent apoptotic pathways in human normal keratinocytes**
Bruno Canguilhem, Anne Pradines, Marie Charvéron, Gilles Favre (Toulouse, FRA)

P606 **Wavelength dependence of cell cycle responses in human melanocytes and melanoma cells following exposure to ultraviolet radiation.**
Christine Kowalczuk, Marisa Priestner, Rick Saunders, Simon Bouffler (Chilton, GBR)

P607 **Role of UV-induced oxidative stress in the regulation of the GTPase RhoB**
Bruno Canguilhem, Sarah Blaquière, Hélène Hernandez-Pigeon, Anne Pradines, Marie Charveron, Gilles Favre (Toulouse, FRA)

P608 **Molecular responses of normal human caucasian melanocytes in culture exposed to simulated solar UV: could melanin and its precursors behave as endogenous photosensitizers?**
Laurent Marrot, Jean-Philippe Belaidi, Christophe Jones, Philippe Perez, Jean-Roch Meunier (Aulnay-sous-Bois, FRA)

P609 **Wavelength dependency of ultraviolet radiation induced skin infiltration of neutrophils**
Peter L. Lee[1], Huib van Weelden[1], Piet L.B. Bruijnzeel[2] ([1]Utrecht, [2]Oss, NED)

P610 **Limitations of UV-A1 high dose therapy**
Helmut Piazena (Berlin, GER)

P611 **The effect of the short wavelength ultraviolet radiation. An extension of biological dosimetry to the UV-C range**
Berces A., Kovacs G., Ronto G. (Budapest, HUN)

P612 **Inactivation of bacteria in platelet concentrates by UVC irradiation**
Harald Mohr, Ute Gravemann, Anette Bayer, Thomas H. Müller (Springe, GER)

P613 **Photo-induced crosslinking of isolated calf-lens proteins and crystallin fractions sensitized by advanced glycation endproducts**
Denis Fuentealba, Eduardo Silva (Santiago, CHI)

P614 **Singlet oxygen-mediated tryptophan oxidation: characterization of potential markers of protein oxidation**
Michelle Gracanin, David I. Pattison, Michael J. Davies (Sydney, AUS)

P615 **Effects of low power laser on plasmatic glycerol: an experimental study in rats**
Halina Camargo Senhorinho, Gerson Linck Bichinho, Munir Antônio Gariba, Percy Nohama (Curitiba, BRA)

P616 **Visible-light-induced respiratory rate increase in cells: searching for a plausible photoacceptor**
A. Remedi, L. di Salvo, G. Cercignani, S. Lucia, G. Colombetti (Pisa, ITA)

P617 **Refinement and use of an *in vitro* model for the measurement of the vitamin D production capacity of sunlight**
Alex Russell McKinley, Michael G. Kimlin, Michael R. Moore (Brisbane, AUS)

P618 **Scattered UV radiation, shade structures and vitamin D**
David J Turnbull, Alfio V Parisi (Toowoomba, AUS)

P619 **First experiences in using broadband instruments and electronic personal dosimeters designed for erythemally effective UV radiation to measure the vitamin D effective UV radiation**
Alois W. Schmalwieser[1], Peter Koepke[2] ([1]Vienna, AUT; [2]Munich, GER)

P620 **Utilising polyphenylene oxide for long term solar UVA dosimetry**
David John Turnbull, Peter W Schouten (Toowoomba, AUS)

P621 **Costa del Salford: emulating summer UV exposure in north-west England**
Richard Kift, Lucy Bunhill, Donald Allan, Marie Durkin, Jacqueline Berry, Lesley E. Rhodes, Ann.R.Webb (Manchester, GBR)

P622 **Clear sky UV simulations in the 21st century based on CCM predictions**
Kleareti Tourpali[1], Alkiviadis Bais[1], Andreas Kazantzidis[1], Neal Butchart[2], Christoph Brühl[3], Martin Chipperfield[4], Martin Dameris[5], M. A. Giorgetta[6], U. Langematz[7], Eva Mancini[8], E. Manzini[9], Gianni Pitari[8], E. Rozanov[10] ([1]Thessaloniki, GRE; [2]Exeter, [4]Leeds, GBR; [3]Mainz, [5]Oberpfaffenhofen, [6]Hamburg, [7]Berlin, GER; [8]L'Aquila, [9]Bologna, ITA; [10]Davos, SUI)

P623 **Abnormal IL-4 and IL-12p70 secretion in stimulated peripheral blood mononuclear cells (PBMC) from patients with Polymorphic Light Eruption (PLE) is unaffected by TL-01 phototherapy**
Alison Blackburn, Sandra M. Winhoven, Paul Balmer, Margaret Brownrigg, Lesley E. Rhodes, Neil K. Gibbs (Manchester, GBR)

P624 **Erythemal sensitivity does not predict UVB-induced epidermal CD1a+ Langerhans' cell loss or caspase-3 activation in Polymorphic Light Eruption (PLE)**
Joanne Tye, Alison Blackburn, Sandra M. Winhoven, Margaret Brownrigg, Lesley E. Rhodes, Neil K. Gibbs (Manchester, GBR)

P625 **Ultraviolet irradiation directly influences the structure of fibrillin microfibrils**
Siobhan M. Reilly, Neil K. Gibbs, Christopher E.M. Griffiths, Rachel E.B. Watson, Michael J. Sherratt (Manchester, GBR)

P626 **Spectrophotometric study of antioxidant and iron binding capacity of selected flavonoid compounds**
George Zonios, Aikaterini Dimou, Dimitrios Galaris (Ioannina, GRE)

P627 **The effects of differently coated titanium dioxide particles on UVA-induced oxidation of biological substrates**
Elisabetta Venditti, Lucedio Greci, Elisabetta Damiani (Ancona, ITA)

P628 **Effects of the flavonoid luteolin on the human sunburn response**
Lien Verschooten[1], Katrien Smaers[1], Lieve Declercq[2], Daniel Maes[2], Marjan Garmyn[1] ([1]Leuven, [2]Oevel, BEL)

P629 **Which hazard weighting function should be used for assessment of eye exposure from dental radiation sources?**
Ellen M Bruzell[1], Bjørn Johnsen[2], Tommy Nakken Aalerud[2], Terje Christensen[2] ([1]Haslum, [2]Østerås, NOR)

P630 **Misuse of light in tooth bleaching**
Ellen M Bruzell[1], Jon E Dahl[1,2] ([1]Haslum, [2]Oslo, NOR)

P631 **Spectroscopic and electrochemical properties of 2-aminophenothiazine in acetonitrile.**
Rolando Oyola[1], Carmelo García[1], Luis Piñero[1], Ileana Nieves[1], Nadya Cruz[1], Rafael Arce[2] ([1]Humacao, [2]San Juan, PUR)

P632 **Photoreactivity of indirubin derivatives**
David Olivier, Samuel Douillard, Thierry Patrice (Nantes, FRA)

P633 **Photoredox processes in synthetic nucleosides: intramolecular photoreactions of ketoprofen-purine dyads**
Cecilia Paris[1], Noureddine Belmadoui[2], María José Climent[1], Susana Encinas[1], Miguel Ángel Miranda[1] ([1]Valencia, ESP; [2]Grenoble, FRA)

P634 **Valence isomerization of hinokitiol under UV irradiation**
Koh-ichi Seki[1], Michiko Maekawa[1], Shigeru Kasahara[1], Shingo Nakamura[2], Toshihiro Okabe[3], Tadamiti Yamamoto[3], Yoshihiro Inamori[4], Naomi Shishido[5], Masao Nakamura[5] ([1]Sapporo, [2]Aomori, [3]Hirosaki, [4]Takatuki, [5]Asahikawa, JPN)

P635 **Diastereoselective photocycloaddition of nucleoside aanalogues with naphthalene**
Koh-ichi Seki[1], Masayuki Takahashi[2], Ken-ichi Nishijima[2], Kazue Ohkura[2] ([1]Sapporo, [2]Ishikari-Tobetsu, JPN)

P636 **The triplet properties of the photocarcinogenic fluoroquinolone antibiotic, lomefloxacin, are not consistent with an energy transfer mechanism for photosensitised thymine dimer formation.**
Kristy Clarke[1], George Truscott[1], David J. McGarvey[1], Neil K. Gibbs[2] ([1]Keele, [2]Manchester, GBR)

P637 **Photosensitization of cholesterol by diaryl ketones in model dyads**
Inmaculada Andreu, Isabel M. Morera, Francisco Boscá, Miguel A. Miranda (Valencia, ESP)

P638 **Photophysical and photochemical characteristics of 3-nitrofluoranthene in solution**
Salvador D Gavalda, Rafael Arce (San Juan, PUR)

P639 **The poor teacher's spectrophotometer for *in vitro* and *in vivo* absorption and fluorescence spectra**
Lars Olof Bjorn (Lund, SWE)

P640 **James Prescott Joule (1818 - 1889): a Manchester son and the father of the international unit of energy**
Sandra M Winhoven, Neil K. Gibbs (Manchester, GBR)

P641 **Nanocluster photocatalytic degradation of some organic pollutants**
Souad Ahmed Elfeky, Alsayed Abdelmajid Alsherbini (Giza, EGY)

P642 **Photoinactivation of bacteria in wastewater by porphyrins. Bacterial β-galactosidase activity and leucine-uptake as methods to monitor the process**
C.M B. Carvalho, A.T.P.C. Gomes, S.C.D. Fernandes, A.C.B. Prata, M.A. Almeida, M.A. Cunha, J.P.C. Tomé, M.A.F. Faustino, M.G.P.M.S. Neves, A.C. Tomé, J.A.S. Cavaleiro, Z. Lin, J.P. Rainho, J. Rocha (Aveiro, POR)

P643 **Photochemical degradation of phenylurea herbicide chlorotoluron**
Sarka Klementova, Martina Zemanova (Ceske Budejovice, CZE)

P644 **Bioluminescent control of sterility**
Martina Bancirova, Hana Fabianova (Olomouc, CZE)

P645 **Kinetic properties of bacterial luciferases in water-organic solvents**
Irina E. Sukovataya, Elisaveta V. Kaykova (Krasnoyarsk, RUS)

P646 **Differences in processing of blue light signals between animal and plant cryptochromes**
Tilman Kottke[1,2], Alex Berndt[3], Helena Breitkreuz[3], Joachim Heberle[1], Margaret Ahmad[4], Eva Wolf[3] ([1]Bielefeld, [2]Juelich, [3]Dortmund, GER; [4]Paris, FRA)

P647 **A blue light inducible two component signal transduction system in the plant pathogen *Pseudomonas syringae* pv. tomato**
Zhen Cao[1], Valentina Buttani[2], Aba Losi[2], Wolfgang Gaertner[1] ([1]Muelheim an der Ruhr, GER; [2]Parma, ITA)

P648 **Towards a more sensitive phytoplankton?**
Cristina Sobrino[1], Patrick J. Neale[2], Robert E. Moeller[3], Jason Porter[4], Jesse Phillips-Kress[2] ([1]Vigo, ESP; [2]Edgewater MD, [3]Oxford OH, [4]Bethlehem PA, USA)

P649 **Mycosporines and Mycosporine-like Amino Acids (MAAs) - an old story but ever exciting**
Manfred Klisch, Donat-P. Häder (Erlangen, GER)

P650 **Short term impact of ultraviolet radiation on photosynthesis of marine diatoms**
Luis Mendía, Marcos Lagunas, E. Walter Helbling (Playa Unión-Rawson, ARG)

P651 **Sun and shade acclimation of the photosynthetic apparatus within single leaves**
Christine Ewers, Wolfgang Bilger (Kiel, GER)

P652 **High light induced ROS and their effects on photoinhibion in *Arabidopsis thaliana* mutants with reduced amount of light harvesting complexes**
Milos Bartak[1], Jaroslav Lang[1], Kristyna Vecerova[1], Pavel Pospisil[2], Petr Ilik[2] ([1]Brno, [2]Olomouc, CZE)

P653 **Why form plants UV-B screening pigments at low temperature?**
Matthias Schultze, Wolfgang Bilger (Kiel, GER)

P654 **Impact of high intensity UV-B radiation on leaf chlorophyll fluorescence and content of ultraviolet-absorbing compounds of cucumber and peppermint plants**
Elżbieta Skórska, Maja Wencierska (Szczecin, POL)

P655 **Morphological and physiological changes in the UV-B irradiated plants of triticale differing in leaf wax presence**
Elżbieta Skórska, Wiktor Szwarc (Szczecin, POL)

P656 **Effect of dehydration stress on delayed luminescence of plant leaves**
Joonho Kang[1], Daeshik Kim[1], Daewoong Jeong[1], Sanghyun Park[2], Kwangsup Soh[2] ([1]Busan, [2]Seoul, KOR)

P657 **Effect of isotope substitution and controlled dehydration on the molecular mobility and photoinduced electron transport reactions of quinone acceptors and multiheme cytochrome c in bacterial photosynthetic reaction center**
Constantine Chamorovsky, Sergei Chamorovsky, Peter Knox (Moscow, RUS)

P658 **Ultraviolet interferential filters for the realization of sensors with spectral responses equivalent to biological action curves**
M.G. Pelizzo, D. Garoli, P. Nicolosi (Padova, ITA)

P659 **Photo-stability of sunscreen products in the full solar spectral range**
D. Garoli, M.G. Pelizzo, P. Nicolosi, A. Peserico, M. Alaibac (Padova, ITA)

Wednesday, September 5, 14.15 – 15.45 **Sports Hall at Founders Complex**

Poster session II

P701 **Porphyrin transport in HaCaT keratinocytes after treatment with 5-aminolevulinic acid or its methyl ester derivative: effect of fumitremorgin C on intracellular porphyrin content, phototoxicity and photogenotoxicity.**
Claire Hill, Nicola J Traynor, Harry Moseley, William Ogilvie, James Ferguson, Julie A Woods (Dundee, GBR)

P702 **Isolation and characterization of squamous carcinoma cells resistant to photodynamic treatment with methyl-aminolevulinic acid.**
V. Rivarola[1], F. Sanz-Rodríguez[2], A. Zamarrón[2], B. Rumie[1], I. Yslas[1], A. Blazquez[2], A. Juarranz[2] ([1]Rio Cuarto, ARG; [2]Madrid, ESP)

P703 **Assessment of the topical photoallergy potential of METVIX cream.**
Douglas Brian Learn[1], Christopher Paul Sambuco[1], Paul Donald Forbes[1], Alan M. Hoberman[1], Jacques Cambrou[2], Franck Chuzel[2] ([1]Horsham PA, USA; [2]Sophia Antipolis, FRA)

P704 **Combined effect of 5-ALA treatment with the use of NF-kB inhibitors for the survival of Glioblastoma Multiforme cells.**
Isabelle Coupienne, Pierre A Robe, Jacques Piette, Sebastien Bontems (Liege, BEL)

P705 **Activation and reorganization of skin keratinocytes and fibroblasts by photodynamic therapy with methyl-aminolevulinic acid**
A. Juarranz[1], F. Sanz Rodríguez[1], A. Blázquez[1], M.C. Iglesias-de la Cruz[1], L. Bagazgoitia[1], J. Cuevas[2], S. González[3,1], P. Jaen[1] ([1]Madrid, [2]Guadalajara, ESP; [3]New York, USA)

P706 **Evaluation of porphyrin production from 5-aminolaevulinic acid by pathogenic microbes**
Ludovic Bourre[1], Francesca Giuntini[2], Ian M. Eggleston[2], Alexander J. MacRobert[1], Michael Wilson[1] ([1]London, [2]Bath, GBR)

P707 **Visible light inactivation of selected bacteria and fungi by modified titania**
Agnieszka Jańczyk[1], Dariusz Mitoraj[1,2], Magdalena Strus[1], Wojciech Macyk[1], Piotr Bogumł Heczko[1], Horst Kisch[2], Grażyna Stochel[1] ([1]Kraków, POL; [2]Erlangen, GER)

P708 **Antibacterial effect of PDT using the cationic photosensitiser**
V. Negrimovsky, V. Mamikonyan, M. Balayan, M. Budzinskaya, F. Fedorov, M. Strakhovskaya, S. Shevchik, V. Loschenov, S. Kuzmin, G. Vorozhtsov (Moscow, RUS)

P709 **Targeted delivery of photosensitizing dyes via conjugation with bacterial peptide derivatives for the photodynamic therapy of bacterial infections**
Ulysses Wilson Sallum, Humra Athar, Sarika Verma, Tayyaba Hasan (Boston MA, USA)

P710 **Photodynamic inactivation of *Allicyclobacillus acidoterrestris* with phenotiazinic dyes**
Janice R. Perussi, Juliana V. Alberice, Roberta M. Maria, Emanuel Carrilho, Hidetake Imasato (São Carlos, BRA)

P711 **Photodynamic effect on *Helicobacter pylori***
M. H. El Batanouny, Rehab M. Amin, E.S. El Gohary, M.K. Ibrahium, M.I. Naga, M.S. Salama (Cairo, EGY)

P712 **Metal-phosphate coordination determines the adsorption and photodynamic activity of sulfonated metallophthalocyanines on phospholipid membranes**
Alina A. Pashkovskaya, Elena A. Sokolenko, Valeri S. Sokolov, Elena A. Kotova, Yuri N. Antonenko (Moscow, RUS)

P713 **Dark and photohemolytic activity of some amphiphilic deuteroporphirin derivatives**
Mikhail V. Malakhov, Galina V. Mansurova, Gelii V. Ponomarev, Andrei V. Reshetnikov, Alexander Ya. Potapenko (Moscow, RUS)

P714 ***In vitro* phototoxicity study using novel phthalocyanines and incoherent light source**
Nil Saydan, Sinem Tuncer, Menaf Ayhan, Devrim Atilla, Ayse Gul Gurek, Mahmut Durmus, Vefa Ahsen (Gebze, TUR)

P715 **Comparative photodynamic effects of three photosensitisers in a human carcinoma cell line**
Alcira Batlle[1], María Gabriela Alvarez[2], Viviana Rivarola[2], Haydée Fukuda[1] ([1]Buenos Aires, [2]Río Cuarto, ARG)

P716 **Preparation and characterization of novel biofunctionalized fluorescent silica nanoparticles and their possibility for photodynamic therapy**
Michihiro Nakamura, Masayuki Shono, Kazunori Ishimura (Tokushima, JPN)

P717 **Photodynamic effect in human epithelial cells of a new methionine-porphyrin conjugate**
M.A.F. Faustino[1], V. Vaz Serra[1], J.P.C. Tomé[1], M.G.P.M.S. Neves[1], A.C.Tomé[1], J.A.S. Cavaleiro[1], F. Sanz-Rodríguez[2], B. Bautista[2], A. Zamarrón[2], A. Blázquez[2], A. Juarranz[2] ([1]Aveiro, POR; [2]Madrid, ESP)

P718 **Synthesis and characterization of phenothiazine- and squaraine-based photosensitizers for photodynamic therapy**
Prakash Sanjeevaiah, Basel Zaitoun, Kenneth W. Olsen, David S. Crumrine (Chicago IL, USA)

P719 **New porphyrin amino acid conjugates: synthesis and photodynamic effect in human epithelial cells**
F. Sanz-Rodríguez[1], A. Zamarrón[1], B. Bautista[1], A. Blazquez[1], A. Juarranz[1], V. Vaz Serra[2], M.A.F. Faustino[2], J.P.C. Tomé[2], M.G.P.M.S. Neves[2], A.C. Tomé[2], J.A.S. Cavaleiro[2] ([1]Madrid, ESP; [2]Aveiro, POR)

P720 **Development of sensitizers based on halogenated tetrapyrroles as new PDT-agents against selected melanoma and adenocarcinoma cells.**
Janusz M. Dąbrowski[1], Carlos J.P. Monteiro[2], Luis G. Arnaut[2], Mariette M. Pereira[2], Krystyna Urbańska[1], Grażyna Stochel[1] ([1]Krakow, POL; [2]Coimbra, POR)

P721 **Metabolic profile and *in vivo* stability of a peptide-conjugated chlorin-type photosensitiser targeting neuropilin-1: interest of pseudopeptides.**
Noémie Thomas[1], Loraine Tirand[1], Denise Bechet[1], Regis Vanderesse[2], François Plénat[1], Céline Frochot[2], François Guillemin[1], Muriel Barberi-Heyob[1] ([1]Vandoeuvre-lès-Nancy, [2]Nancy, FRA)

P722 **Investigations of photosensitizer peptide conjugates for increased PDT efficacy**
Alexis Lynn Reid, Katie Latulippe, Roy Pottier, Eva Gudgin-Dickson, James T.C. Wojtyk (Kingston, CAN)

P723 **Chlorin e6 polyvinylpyrrolidone selectively accumulates and causes photodynamic damage in human bladder carcinoma: from chick chorioallantoic membrane model to clinical patients**
William WL Chin, Weber KO Lau, Paul WS Heng, Pei Li Lim, Ramaswamy Bhuvaneswari, Malini Olivo (Singapore, SIN)

P724 **The membrane localization of tetrapyrrolic photosensitizers as a determinant of their photodynamic efficiency. A comparison between Chlorin e6 and mTHPC**
Halina Mojzisova, Stéphanie Bonneau, Christine Vever-Bizet, Daniel Brault (Paris, FRA)

P725 **A preliminary study of photodynamic effect on matrix metalloproteinases activity in mesothelioma cell lines**
Meltem Goksel Dizge, Nil Saydan, Mahmut Durmus, Ayse Gul Gurek, Vefa Ahsen (Gebze, TUR)

P726 **Disorganisation of cytoskeleton and differential cell adhesion in cells resistant to photodynamic therapy**
A. Casas[1], F. Sanz-Rodríguez[2], G. Di Venos[1], A. Batlle[1], J.C. Stockert[2], A. Juarranz[2] ([1]Buenos Aires, ARG; [2]Madrid, ESP)

P727 **Optimizing hypericin mediated photodynamic therapy downregulates the expression of angiogenic proteins in bladder carcinoma**
Ramaswamy Bhuvaneswari, Gan Yap-Yik Yuen, Soo Khee Chee, Malini Olivo (Singapore, SIN)

P728 **Characterization by MALDI-FTICR mass spectrometry of target protein potentially implied in photodynamic therapy**
Benoit Maunit[1], Marc Dodeller[1], David DA Silva[1], Lina Bezdetnaya[2], François Guillemin[2], Jean François Muller[1] ([1]Metz, [2]Nancy, FRA)

P729 **The effect of photodynamic therapy on mesothelioma cell lines**
Murat Dogru, Sinem Tuncer, Devrim Atilla, Ayse Gul Gurek, Mahmut Durmus, Vefa Ahsen, Nil Saydan (Gebze, TUR)

P730 **Modulation of photodynamic injury of crayfish neuronal and glial cells by neurotrophic factors**
Andrej V.Lobanov, Anatoly B. Uzdensky (Rostov-on-Don, RUS)

P731 **Zn(II)phthalocyanine-mediated mitotic catastrophe on HeLa cells**
Santiago Rello-Varona, Juan Carlos Stockert, Magdalena Cañete, Angeles Villanueva (Madrid, ESP)

P732 **Intracellular localisation of mTHPC and effect of photodynamic therapy in cells of the mammalian peripheral nervous system**
K.E. Wright[1], A.J. MacRobert[2], J.B. Phillips[1] ([1]Milton Keynes, [2]London, GBR)

P733 **LDL as delivery system of hypericin in U-87MG glioma cell line: sub-cellular distribution of hypericin and efficiency of PDT**
Zuzana Nadova[1], Veronika Huntosova[1], Anton Mateasik[2], Jaromir Mikes[1], Pavol Miskovsky[1] ([1]Kosice, [2]Bratislava, SVK)

P734 **The effects of increasing oxygen concentrations in PDT on cell survival and lipid peroxidation in different cancer cell lines**
Astrid Hjelde, Odrun A. Gederaas, Hans E. Krokan, Alf O. Brubakk (Trondheim, NOR)

P735 **Surface-enhanced Raman and fluorescence spectroscopy of hypericin in the presence of LDL and phosphatidylcholine**
Gejza Lajos[1], Santiago Sanchez-Cortes[2], Daniel Jancura[1], Jose Vicente Garcia-Ramos[2], Pavol Miskovsky[1,3] ([1]Kosice, [3]Bratislava, SVK; [2]Madrid, ESP)

P736 **Photochemical Internalization (PCI) with adenovirus targeted to the epidermal growth factor receptor (EGFR)**
Anette Bonsted, Håvard Kirkevold, Inger Lise Aagaard, Kristian Berg (Oslo, NOR)

P737 **Kinetics of the interactions of hypericin with low density lipoproteins**
Luboslava Buriankova[1], Veronika Huntosova[1], Diana Buzova[1], Daniel Jancura[1], Pavol Miskovsky[1,2] ([1]Kosice, [2]Bratislava, SVK)

P738 **Serum albumin as carriers for phthalocyanines**
Emilio Alarcón[1], Ana M. Edwards[1], Angélica García[1], Marcelo Muñoz[1], Maureen Fajardo[1], Giulio Jori[2] ([1]Santiago, CHI; [2]Padova, ITA)

P739 **Oligonucleotides binding constants of photosensitizer dyes and of alkylating quinones and nitroarenes with potential use in photodynamic therapy in hypoxic environments**
Yisaira Diaz-Espinosa[1], Rafael Arce[2], Antonio Alegria[3] ([1]San Juan, [2]Rio Piedra, [3]Humacao, PUR)

P740 **Full determination of PDT parameters for optimizing PDT applications**
Lilian Tan Moriyama, Priscila Fernanda Campos de Menezes, José Dirceu Vollet Filho, Juliana Ferreira, Vanderlei Salvador Bagnato (São Carlos, BRA)

P741 **Photodynamic therapy for cancer cells using mixed wavelength LEDs**
Toshiyuki Kosobe[1], Yasushi Takei[1], Xuepeng Qiu[2], Norimichi Kawashima[1], Yoshikazu Tokuoka[1] ([1]Yokohama, JPN; [2]Changchun, CHN)

P742 **Treatment of acne: the role of photodynamic therapy and micropeeling**
Marcello Monti, Stefania Motta (Milano, ITA)

P743 **Spectroscopic evaluation of near-IR quantum dots for cancer diagnosis**
Elnaz Yaghini, Arthur Iga, Alexander M. Seifalian, Alexander J. MacRobert (London, GBR)

P744 **Possibilities for real time dosimetry for PDT**
Priscila Fernanda Campos de Menezes (São Carlos, BRA)

P745 **Early diagnosis of cancers by fluorescence imaging of cytologic slides**
Karine Steenkeste[1], Ariane Deniset[1], Sandrine Lécart[1], Sandrine Lévêque Fort[1], Pascal Eschwege[2], Sophie Ferlicot[2], Michèle Fabre[2], Basile Maldant[3], Marie-Pierre Fontaine-Aupart[1] ([1]Orsay, [2]Le Kremlin Bicêtre, [3]Bièvres, FRA)

P746 **The photodynamic action *vs* catechol**
Martina Bancirova, Jana Jarošová, Veronika Borská (Olomouc, CZE)

P747 **Products of photosensitizer photooxidation are responsible for systemic immunosuppression**
Alla A. Kyagova, Elena A. Kozhinova, Lyudmila A. Kozir, Galina V. Mansurova, Zoya I. Moshnina, Gelii V. Ponomarev, Alexander Ya. Potapenko (Moscow, RUS)

P748 **Fluorescence imaging for demarcation of Basal Cell Carcinoma tumor borders**
Claes D. Enk[1], Joseph Alcalay[2], Clemens Fritsch[3], Thomas Ruzicka[3], Ronen Alkalay[1] ([1]Jerusalem, [2]Tel Aviv, ISR; [3]Munich, GER)

P749 **Induced phototoxicity of cell culture media**
Thierry Patrice, David Olivier, Samuel Douillard (Nantes, FRA)

P750 **Phototoxicity is not associated with Rose Bengal-photosensitized tissue bonding**
Min Yao, Kenneth Bujold, Robert Redmond, Irene Kochevar (Boston MA, USA)

P751 **Photochemical reactions of bilirubin and folic acid in different solutions**
A.Vorobey[1], V.Plavsky[1], P.Vorobey[1], A.H.Steindal[2], J.Moan[2] ([1]Minsk, BLR; [2]Oslo, NOR)

P752 **Differentiation of curcumin phototoxicity in cells and bacteria by change in pharmaceutical preparation**
Tone Haukvik[1,2], Ellen M. Bruzell[2], Inger Sofie Dragland[2], Else Morisbak[2], Hanne Hjorth Tønnesen[1] ([1]Oslo, [2]Haslum, NOR)

PL101

Interactions between drug excited states and proteins or DNA

Virginie Lhiaubet-Vallet

Instituto de Tecnología Química UPV-CSIC, Universidad Politécnica de Valencia, Valencia, Spain

Drug-biomolecule interactions in the excited state are relevant from a photobiological point of view as they can be correlated with a number of photosensitization disorders such as photocarcinogenicity, photoallergy, phototoxicity, etc. These processes are promoted by the drug chromophores absorbing in the UVA- or visible fractions of solar spectrum. The resulting excited states may act as photosensitizers, mediating chemical transformations of key biomolecules (lipids, proteins and/or nucleic acids). Moreover, the increasing trend toward the investigation of biomolecules photosensitization can be related with the number of drugs absorbing UV-light, and thus, able to induce important photobiological damages.

In this presentation, the non steroidal antiinflammatory 2-arylpropionic acids and the antibacterial fluoroquinolones have been selected as typical examples of photoactive drugs. They have been submitted to systematic studies of their steady-state and time-resolved photophysical properties alone and in the presence of proteins, nucleic acids or their simple building blocks.

In a first part, stereodifferentiation in photophysical and photochemical processes in the presence of biomolecules have been considered. The detailed knowledge of these stereodifferentiating interactions in the excited states appears essential for the design of new chiral therapeutic agents aimed at improving the benefit/risk ratio by decreasing the photosensitivity side effects. Despite the significance of such studies, the photobiological properties of chiral drugs remain practically unexplored until now.

Then, another important point will be addressed and concerns thymine cyclobutane dimers formation. This is one of the most important lesions formed by direct UV-exposure of DNA. Besides, it can also be formed upon DNA-photosensitization by triplet-triplet energy transfer from a drug excited state to thymine. Thus, the triplet energy of thymine in DNA is a critical parameter that has been determined here in a more accurate way or, more precisely, the minimum value of the triplet state energy for a photosensitizer to produce thymine lesions in DNA has been fixed.

IL102

Absorption of UV radiation by DNA helices: collective effects and time-resolved studies

D. Markovitsi, D. Onidas, T. Gustavsson, F. Talbot, S. Marguet, E. Lazzarotto

Laboratoire Francis Perrin CEA-CNRS URA 2453, Gif-sur-Yvette, France

DNA bases within double helices may adapt a collective behaviour when exposed to UV radiation. In this talk we will illustrate that such cooperativity intervenes at the very first steps following excitation at 267 nm. Our studies show the existence of excited states delocalized over several bases (excitons) and auto-ionizing states. They were performed for double helices $(dA)_n.(dT)_n$ and $(dAdT)_n.(dAdT)_n$ dissolved in phosphate buffer according to specifically developed experimental protocols.[1]

Delocalization of excited states was evidenced by probing the intrinsic fluorescence of the double strands induced by femtosecond pulses. Emission was detected by fluorescence upconversion and time-correlated single photon counting over a large time domain (100 fs – 100 ns).[2-5] The ensemble of our observations (fluorescence decays, fluorescence anisotropy decays, time-resolved spectra, steady-state absorption and fluorescence spectra) were interpreted in terms of exciton states populated directly followed by energy transfer occurring in less than 100 fs. The wavelength dependence of the fluorescence anisotropy decays reveals that emission arises from a variety of excited states. Interestingly, the portion of emitting excitons compared to monomer emission increases with increasing the size of the double helix.

Moreover, ionization of model helices was studied by nanosecond flash photolysis.[6] The ejected electrons were quantified by probing the absorption of the hydrated electron concentration. Its variation with the absorbed laser intensity proved that one photon ionization takes place. The absorption spectra of the duplexes on the microsecond time-scale correspond to the adenine deprotonated radical, formed in concentrations comparable to that of the hydrated electron. The quantum yield for one photon ionization (*ca.* 10^{-3}) is higher by at least one order of magnitude than that of dAMP.

[1]J. Photochem. Photobiol. A: Chem., 2006, 183, 1. [2]ChemPhysChem, 2003, 3, 303. [3]J. Am. Chem. Soc., 2005, 127, 17130. [4]Nature, 2006, 441, E7. [5]Photochem. & Photobiol. Sci., 2007, in press. [6]J. Phys. Chem. B, 2006, 110, 11037.

IL103

Photochemistry of 5-halouracil-containing DNA

Hiroshi Sugiyama

Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto, Japan

The photoreactivities of 5-halouracil-containing DNA have widely been used for analysis of protein–DNA interactions, and have recently been used for probing charge transfer processes along DNA. We recently discovered that photoirradiation of ^{Br}U-substituted DNA efficiently produced 2'-deoxyribonolactone at 5'-(G/C)AA^{Br}U^{Br}U-3' and 5'-(G/C)A^{Br}U^{Br}U-3' sequences in duplex DNA. Using synthetic oligonucleotides, we found that similar photoreactivities were maintained at the 5'-(G/C)AA^{Br}UT-3' sequence, providing ribonolactone as a major product. In this paper, the photoreactivities of various oligonucleotides possessing the 5'-^{Br}UT-3' sequence were examined to elucidate the essential factors of this photoreaction. HPLC product analysis indicated that the yield of 2′-deoxyribonolactone largely depends on the ionization potential of the purine derivatives located 5'-upstream of 5'-^{Br}UT-3', as well as the electron-donating ability of their pairing cytosine derivatives. These results clearly suggest that the photoinduced charge transfer from the G 5'-upstream of 5'-^{Br}UT-3' sequence, in the same strand and the complementary strand, initiates the reaction. To examine the role of intervening A/T base pair(s) between the G/C and the 5'-^{Br}UT-3' sequence, the photoreactivities of a series of oligonucleotides with different numbers of intervening A/T base pairs were examined. The results revealed that the hotspot sequence consists of the electron-donating G/C base pair, the 5'-^{Br}UT-3' sequence as an acceptor and an appropriate number of A/T base pairs as a bridge for the charge transfer process.

IL104

Structural investigation of cyclobutane pyrimidine dimers in complex with DNA photolyase and RNA polymerase II

Thomas Carell

LMU Munich, Department of Chemistry and Biochemistry, Munich, Germany

Our genome is constantly damaged by various exogenous and endogenous events. These lesions interfere with the normal transcription and replication events. In the lecture we will describe the chemical synthesis of oxidative DNA lesions, DNA lesions which are formed due to UV-irradiation, and of cisplatin lesions. We discuss how these lesions are incorporated into oligonucleotides using solid phase chemistry. The obtained DNA strands, which contain one of these lesions at a defined site, were subsequently used to create co-crystal structures with various proteins influenced by such DNA lesions. We will show structures of RNA Pol II[1] in complex with CPD lesions, of various DNA polymerases[2] in complex with DNA containing oxidative lesions and of the low fidelity polymerase Pol eta in complex with a

cisplatin lesion as well as of repair enzymes[3] in complex with damaged oligonucleotides. From these structures and correlated biochemistry we learn how nature achieves to couple transcription to DNA repair and how the mechanism of translesion synthesis allows cells to replicate DNA in the presence of lesions.[4]

[1]F. Brückner, U. Hennecke, T. Carell, P. Cramer, Science 2007 in press. [2]G.W. Hsu, M. Ober. T. Carell, L. Beese, Nature 2004, 431, 217-221. [3]A. Mees, T. Klar, P. Gnau, U. Hennecke, A.P.M. Eker, T. Carell, L.-O. Essen, Science 2004, 306, 1789-1793. [4]Unpublished data

IL105

Specific features of the formation and repair of far-UV induced dimeric photoproducts at the four bipyrimidine dinucleotides within isolated and cellular DNA

Thierry Douki[1], Stephane Mouret[1], Evelyne Sage[2], Fabien Joux[3], Sabine Matallana Surget[3], Jarah A. Meador[3], Jean Cadet[1]

[1]DRFMC/SCIB/LAN; CEA-Grenoble, Grenoble, France; [2]Institut Curie, Orsay, France; [3]Laboratoire d'Océanographie Biologique, Banyuls-sur-Mer, France

Solar UV radiation is a major DNA damaging agent that induces dimerization of adjacent pyrimidine bases. The resulting lesions are either cyclobutane pyrimidine dimers (CPD), pyrimidine (6-4) pyrimidone photoproducts (64PP) or their Dewar valence isomers. Using a highly specific method, liquid chromatography coupled to tandem mass spectrometry, we first reassessed the distribution of these lesions at the four possible pyrimidine dinucleotides within isolated calf thymus DNA exposed to UVB and UVC radiation. TT and TC were the most photoreactive sites, while a lower dimerization yield was observed at CT and CC. The ratio between the yields of 64PP and CPD drastically depends on the pyrimidine dinucleotide, the largest ratio being observed for TC dinucleotide. This distribution of photoproducts was found to be only affected by drastic irradiation conditions such as denaturation, high temperature, low ionic strength or dehydration. The GC content of the DNA used was also found to strongly modify the relative frequency of the different photoproducts. The distribution of UV-induced DNA damage observed in calf thymus DNA was compared to that observed in cells. A very similar distribution was obtained in mammalian cell lines, primary cultured skin cells and whole human skin. In the latter case, the frequency of CPDs and 64PPs was 20 times lower than in cultured keratinocytes, reflecting the photoprotection afforded by the skin. Experiments in cultured cells also showed that UVA was very effective at converting 64PPs into their Dewar isomers. Indeed, the Dewar isomers were never observed in cells or in isolated DNA exposed to low UVB doses. In contrast, the frequency of Dewar isomers was higher than that of 64PPs in CHO cells exposed to simulated sunlight (5% UVB and 95% UVA). We then investigated how differences in the chemical structure of the photoproducts could influence their repair rate, in primary culture of human skin cells. 64PPs and their Dewar isomers were very rapidly removed, with the same efficiency at TC and TT sites. This limited effect of the nature of the modified pyrimidines on the repair rate did not apply to CPDs. Indeed, the two main photoproducts, TT and TC CPDs, were repaired almost equally slowly. However, CT CPD was found to be removed much faster than other CPDs. CC CPD exhibited an intermediate repair rate. These repair features may be correlated at least partly with UVB mutation spectra that only rarely exhibit mutagenic events at CT sites.

OC106

Induction and repair of UV-induced DNA damage in *Deinococcus radiodurans*

Petra Rettberg[1], Ulrike Pogoda de la Vega[1], Thierry Douki[2], Jean Cadet[2], Günther Reitz[1]

[1]DLR, Institute of Aerospace Medicine, Radiation Biology Division, Linder Höhe, 511477 Köln, Germany; [2]DRFMC/SCIB/LAN; CEA-Grenoble, Grenoble, France

Deinococcus radiodurans belongs to one of the most ionizing radiation resistant families of bacteria. Despite intensive research only typical prokaryotic DNA repair pathways could be identified so far. Our research focuses on the UV-induced repair pathways. In previous studies we showed that this non-spore-forming bacterium also exhibits a broad resistance to monochromatic (254 nm) and polychromatic UV radiation (200 – 400 nm). Since solar UV radiation is an important environmental parameter, all organisms had to develop enzymatic repair pathways for UV-induced DNA damage. By analyzing the distribution pattern of UV-induced bipyrimidine dimers in DNA repair-defective *D. radiodurans* mutants (UVs78, uvrA1 uvsE, and 1R1A, recA) in comparison to the wild-type strain R1, we assume that *D. radiodurans* encodes an as yet undefined repair pathway which operates apparent only when the nucleotide excision repair (NER) and homologous recombination (HR) pathways are inactivated. For this purpose we first monitored the post-irradiation recovery profile of the UVC-exposed mutant strains and wild-type strain R1 by HPLC-MS/MS analysis of the main DNA photoproducts. The NER efficiently removes both cyclobutane pyrimidine dimers (CPD) and (6-4) bipyrimidine adducts. HR assists removal of CPD from UV-irradiated DNA while playing a minor role in the removal of (6-4) adducts. Though the NER is not present in NER-deficient mutant UVs78, up to 10 % of both bipyrimidine photoproduct types have been shown to be equally repaired. Hence, an additional pathway must be available to contribute to the removal of (6-4) adducts. In this context it is interesting to note, that using DNA microarrays for gene expression profiling, we found in addition to the expected induced genes of the uvr-complex that one of the main induced genes is DR1762, a gene with unknown function that resides in a gene cluster with resolvase, a recombinase. Ongoing analysis of the microarray data are promising, since amongst others, genes that have been induced post desiccation and upon ionizing radiation exposure (data of the working group of J. R. Battista) have been shown to be up-regulated as well.

Acknowledgement: We would like to thank the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for funding this project RE 1574/1-1/2. The grant of this project enabled one of the authors, U. Pogoda de la Vega, to accomplish her doctoral thesis.

OC107

Towards time-resolving electron transfer between different tryptophan residues during DNA photolyase activation

Martin Byrdin[1], Andre P.M. Eker[2], Agathe Espagne[1], Sandrine Villette[1], Klaus Brettel[1]

[1]CEA Saclay, iBiTec-S, Gif sur Yvette, France; [2]Erasmus University Medical Centre, Rotterdam, Netherlands

DNA photolyase repairs UV induced single strand lesions, mainly cyclobutane pyrimidine dimers (CPD). To this end, upon excitation by blue/near UV light, the doubly reduced FAD cofactor ($FADH^-$) transfers an electron onto the dark-bound substrate (CPD). After successful repair, the electron is thought to return to the flavin. In isolated photolyase and possibly also after oxidation *in vivo*, the flavin may be found, however, in the semireduced radical state FADH° and needs to be reduced to re-establish catalytic competence. There exists a separate light induced reaction to achieve this, so called photoactivation. Here, the flavin radical absorbs a visible photon and then, in its excited state, extracts an electron from a nearby tryptophan (W382 in *E.coli*). Subsequently, this electron hole travels *via* another tryptophan residue (W359) to

a third one at the protein surface (W306). The W306 radical deprotonates and is then reduced by an external donor or by $FADH^-$. In previous years, we managed to time-resolve these last steps as well as the first one (30 ps) by flash absorption spectroscopy. The inter-tryptophan electron transfer steps, by contrast, could not be detected, as they don't give rise to net absorption changes.
To overcome this difficulty, we make use of the fact that polarized excitation (by a pulsed laser) induces a preferential axis (that of the excited transition of FADH°) in the *a priori* unoriented sample (photoselection), and that the transition dipoles of oxidized W359 and W306 form different angles with this axis (known from the crystal structure). Thus, polarized detection should allow distinguishing between these two chemically identical residues in their transiently oxidized state. To demonstrate this, we replaced W306 by redox inert phenylalanine, thus pruning the electron transfer chain behind W359. We show that the resulting transient absorption polarization pattern at 10 nanoseconds after excitation is in line with the orientation of W359 but well different from wild type photolyase where in the same time scale W306 is oxidized. These results pave the way towards monitoring inter-tryptophan electron transfer in photolyase.

IL108

ALA-PDT cell death: real time imaging of subcellular processes

Zvi Malik, Dana Darvish, Aryeh Weiss
Bar Ilan University, Ramat Gan, Israel

ALA-PDT was investigated by live cell time lapse microscopy to reveal cell death subcellular processes in B16 melanoma cells during a an interval of 60 minutes. The live imaging was targeted to monitor every 2 sec the time dependent PpIX synthesis and photo-bleaching upon 410nm light irradiation. Singlet oxygen generation in the ALA treated cells was revealed by using DCFH-DA, a fluorogenic probe for detecting oxidative stress. Membrane integrity during ALA-PDT was revealed by using the anionic sulforhodamine-B dye which is excluded from intact cells. Phase contrast microscopy was used to visualize the real time quantification of single cell structural damage, cell movement and nuclear shape-volume kinetics. The cytoskeletal damage during PDT was followed-up by live fluorescence microscopy of tubulin-YFP in the B16 trasfected cells.
Increasing ALA incubation time increased PpIX synthesis and accumulation in the cells and lead to higher singlet oxygen yield during PDT. Loss of membrane integrity observed by live confocal imaging of Sulforhodamine-B diffusion into the cytosol due due to direct damage to membrane proteins was observed. In parallel a rapid cease in cell locomotion and cell morphological dynamics was accompanied by nuclear swelling due to water chanells damage and loss in regulation of water exchange of was seen time dependently with light exposure. The quatitaive data was calculated by novel computational analysis and will be presented. Live microscopy showed the gradual damage to the organization of microtules network depicted by disaggregation of the tubulin-YFP polymers and monomer spread into the cytosol, parallel to the morphological paralysis and outer membrane leakage. The present approach of integrative live real-time microscopy and quatitative data analysis revealed the dynamics of ALA-PDT cellular processes which shed light on the multiple systems affected by the generation singlet oxygen on the sub-cellular level. ALA-PDT affects immediately following light irradiation and singlet oxygen generation the outer membrane, the cytoskeleton and the nucleus which in turn changes morphological dynamics and the ion-water balance leading to cell death.

IL109

Mechanisms underlying light fractionated PDT using porphyrin precursors

Henriette S. de Bruijn[1], Ellen R.M de Haas[2], Henricus J.C.M. Sterenborg[1], H A Martino Neumann[2], Dominic J Robinson[2]
[1]Center for Optical Diagnostics and Therapy, Department of Radiation Oncology, Rotterdam, The Netherlands; [2]Department of Dermatology, Erasmus University Medical Center, Rotterdam, The Netherlands

PDT of sBCC using topical 5-aminolevulinic acid and a light fluence of 75 to 100 J cm^{-2} yields unsatisfactory long term results. In several animal models, we have shown that fractionated illumination with two light fractions separated by long dark intervals (> 1 h) results in significantly more response than a single illumination. Response is further enhanced if the fluence of the first light fraction is reduced while the cumulative fluence is maintained. The exact mechanism behind this increase in effectiveness is unclear. Here we have determined if the enhanced effect is also evident in clinical ALA-PDT of sBCC while investigating the details of the mechanism behind the increase in response in animal models using both ALA and m-ALA. We compared the response of sBCC to a single illumination and two-fold illumination scheme in which two light fractions of 20 and 80 J cm^{-2} are performed 4 and 6 hours after the application of a single dose of 20% ALA. 154 patients with a total of 505 primary sBCC, into two groups. 243 lesions were treated using a single illumination of 75 J cm^{-2} at a fluence rate of 50 mW cm-2. Fractionated PDT, at the same fluence rate was performed on 262 lesions. Clinically the CR after a two-fold illumination scheme is significantly greater than that following a single light fraction (P = 0.002, log-rank test). Twelve months after therapy, CR after a two-fold illumination is 97% whereas that after a single illumination is below 89%. Response rates were sustained 24 months after therapy using fractionated illumination (96% vs 86% CR). Our data from animal studies show that the PDT dose delivered in the first light fraction is critical and that dark intervals > 1h were necessary for a significant increase in response. We were unable to show any increase in effectiveness due to light fractionation using m-ALA under similar experimental conditions. Also we have not been able to demonstrate any enhanced response of cells in-vitro using either ALA or m-ALA and light fractionation. Together these data suggest that the localisation of PpIX in-vivo after the administration of ALA may be a critical parameter in the mechanism behind the response of tissues to light fractionated ALA-PDT.

IL110

Detection of urinary bladder cancer with, flow cytometry and hexaminolevulinate in urine samples

Beata Čunderlíková[1], Rolf Wahlqvist[2], Aasmund Berner[1], Vlada Vasovič[1], Trond Warloe[3], Jahn M. Nesland[1], Qian Peng[1]
[1]Department of Pathology, Rikshospitalet-Radiumhospitalet Medical Center, University of Oslo, Oslo, Norway; [2]Oslo Urological University Clinic, Aker University Hospital, Oslo, Norway; [3]Department of Surgical Oncology, Rikshospitalet-Radiumhospitalet Medical Center, University of Oslo, Oslo, Norway

Objectives: Urinary bladder urothelial carcinoma is diagnosed by a combination of cystoscopy and biopsy, and with cytology as a valuable additional technique. The accuracy of cytologic diagnosis depends on the experience of a cytologist and can inevitably vary from one cytologist to another. There is a need for an easy, reliable and objective diagnostic method. In the present study a new method was designed for the detection of bladder cancer cells in urine.
Methods: Flow cytometry was utilized to detect protoporphyrin IX in an artificial model consisting of normal urinary bladder transitional epithelial cells from healthy volunteers' urine and an established human urinary bladder carcinoma cell line, TCCSUP,

after incubation with hexaminolevulinate. In addition, urine samples from 19 patients with histopathologically confirmed superficial bladder cancer were examined.
Results: Incubation of normal urinary bladder transitional epithelial cells or TCCSUP cells with hexaminolevulinate for 1 h resulted in a production of protoporphyrin IX only in the TCCSUP cells. Incubation of a mixture of normal urinary bladder transitional epithelial cells and TCCSUP cells with hexaminolevulinate gave rise to a separated subpopulation of cells with protoporphyrin IX fluorescence. After cell sorting by flow cytometry the protoporphyrin IX-containing subpopulation of cells was confirmed by cytologic examination to be TCCSUP cells. It was possible to detect 5% TCCSUP cells in the mixture of normal urinary bladder transitional epithelial cells/TCCSUP cells. In order to test the feasibility of the method in the clinic diagnosis, urine samples from patients with bladder cancer were also measured with comparable, although preliminary and limited, results to those of cytological examination.
Conclusions: The preliminary results show that the technique may be feasible for the detection of bladder cancer cells in urine with possible advantages of simplicity, reliability and objectivity.

OC111
Photodynamic therapy using topically applied hypericin: comparative effect with methyl-aminolevulinic acid on UV induced skin tumors

Annelies Boiy, Rik Roelandts, Peter de Witte
K.U. Leuven, Leuven, Belgium

Hypericin, a pigment of *Hypericum* species, is under consideration as a topical photodynamical agent. We developed a formulation (0.1% hypericin in a gelcream containing ethanol) that after application under occlusion, during 24 h enabled the penetration of hypericin in the whole of UV induced skin lesions. This suggests that hypericin may provide a therapeutic benefit when targeted to certain skin diseases. The formulation was used to evaluate the efficacy of topical hypericin-PDT in a mouse model for actinic keratosis (AK). The penetration characteristics of hypericin were studied in detail and the tumor selectivity was determined. Additionally, all parameters were compared to methyl-aminolevulinic acid (Me-ALA) as this precursor of protoporphyrin IX (PpIX) is typically used in the clinic for the treatment of AK.
After topical application of hypericin and Me-ALA on separate skin lesions for 24 h and 4 h respectively, analysis of the surface fluorescence demonstrated lesional/normal skin ratios to be 1.82 and 1.67. Microscopically, hypericin fluorescence was found to be mainly located in the stratum corneum. A low but relatively homogenous fluorescence was observed throughout the whole lesional epidermis and dermis yet the intensity was lower than in normal skin. The distribution of PpIX fluorescence was demonstrated to be homogenous over the lesional epidermis, moreover fluorescence levels were increased 2.2-fold as compared to normal skin. Following a single hypericin-PDT treatment (20 mW/cm², 40 J/cm²) 44% of the lesions displayed a total response, 22% a partial response (reduction of the lesional diameter) and 33% of the lesions did not respond to the treatment. After Me-ALA PDT 80% of the lesions displayed a total response and 20% a partial response. The clinical and histological consequences of hypericin-PDT and Me-ALA PDT in responsive lesions were found to be similar yet the effects of Me-ALA were more pronounced. Analysis of treated skin sites revealed full necrosis of the tumors 24 h post-PDT with a marked immune infiltrate present in the dermis. A scab developed after 2 to 4 days which was sloughed off about 7 days after exposure. Afterwards, a red contracted atrophic scar could be observed that progressively healed, leaving a slight impression after 3 weeks. In conclusion, we demonstrated the efficacy of topical hypericin-PDT for the treatment for AK in a mouse model.

OC112
Orthotopic animal models for oncologic photodynamic therapy and photodiagnosis

Marie Ange D'Hallewin, Lina Bezdetnaya, Henri Pierre Lassalle, François Guillemin
Centre Alexis Vautrin CRAN, Nancy University, CNRS UMR 7039, Vandoeuvre les Nancy, France

Photodynamic Therapy is a complex treatment modality where a large array of factors can influence therapeutic outcome. Vascularization, vessel permeability, oxygenation and light distribution in the tissue as well as immune response play a key role in the photodynamic process. Each of these factors can be influenced by the choice of the animal model. It is therefore of the utmost importance to chose an appropriate model for pre clinical oncologic PDT studies. Heterotopic models are easy to reproduce and monitor tumor growth and response to treatment and can be useful to answer specific questions. However, since many factors such as vascularization and microenvironment are different from the native organ, they can not be used as a representative model for PDT in the clinic. Orthotopic tumor models present the closest resemblance to the clinical situation with regard to all the elements involved in PDT. Even then, some existing models have to be adapted in order to exhibit the same features as observed during clinical PDT. We present here a brief organ specific overview of the different orthotopic animal models that can be used for in vivo photodynamic therapy studies.

OC113
Liposomes of aminolevulinic acid and aminolevulinic acid-esters for photodynamic therapy

Alcira Batlle[1], Gabriela Di Venosa[1], Adriana Casas[1], Laura Hermida[1,2], Haydée Fukuda[1,3], María Victoria Defain[1,4], Alexander MacRobert[1,5]
[1]CIPYP - CONICET - UNIV. BUENOS AIRES, Buenos Aires, Argentina; [2]CEQUIPE - INTI - Buenos Aires, Buenos Aires, Argentina; [3]Depto Química Biologica - FCEN, UBA, Buenos Aires, Argentina; [4]CEQUIPE - INTA Buenos Aires, Buenos Aires, Argentina; [5]National Medical Laser Center, UCL Medical School, London, Great Britain

Introduction: Photodynamic Therapy (PDT) is a non-thermal technique for inducing tissue damage with light following administration of a light-activated photosensitising drug which can be selectively retained in malignant or diseased lesions relative to normal adjacent tissue. The use of endogenous Protoporphyrin generated through the heme biosynthetic pathway after administration of 5-aminolevulinic acid (ALA) has led to many applications in photodynamic therapy (PDT).
We designed liposomes of different compositions to improve delivery of ALA and its esterified derivatives ALA-Hexyl ester (He-ALA) and ALA-Undecanoyl ester (Und-ALA) for its use in Photodynamic Therapy (PDT).
Materials and Methods:We employed egg yolk phosphatidyl choline (PC), egg lecithin phosphatidic acid (PA) and egg yolk lecithin phosphatidyl glycerol (PG) in the composition of the liposomes. We prepared small unilamellar vesicles of PC, PC:PG (80:20) or PC:PA (80:20) containing ALA or derivatives and purified by a minicolumn centrifugation method.
Results: Liposomes of 100% PC resulted in percentages of entrapment of around 6% for 2 mM ALA, 13% for 2mM He-ALA and 51% for 2 mM Und-ALA. Addition of PC or PG to the formulation, resulted in increased rates of entrapment: 19% for 2mM ALA, 69% for 2 mM He-ALA and 87% for 2 mM Und-ALA in PC:PG (80:20) and 21% for 2mM ALA, 60% for 2 mM He-ALA and 86% for 2 mM Und-ALA in PC:PA (80:20). Higher concentrations of ALA or derivatives resulted in lower percentages of entrapment. The three formulations containing ALA or derivatives were stable up to 1 week upon storage at 4°C. Incubation of empty liposomes of the three compositions with ALA

or its derivatives resulted in a significant entrapment, showing diffusion of the molecules into the vesicles.
Conclusions: Increased entrapment into liposomes of different composition of ALA-esters as compared to ALA, as well as stability upon dilution with cell culture, have been demonstrated.

OC114

Novel 5-aminolaevulinic acid peptide prodrugs for photodynamic therapy: synthesis, characterisation, and cellular accumulation studies

Francesca Giuntini[1], *Ian M. Eggleston*[1], *Ludovic Bourre*[2], *Alexander J. MacRobert*[2], *Michael Wilson*[3]
[1]*Department of Pharmacy and Pharmacology, University of Bath, Bath, United Kingdom;* [2]*National Medical Laser Center, Division of Surgical and Interventional Sciences, Royal Free and University College Medical School, University College London, London, United Kingdom;* [3]*Division of Microbial Diseases, UCL Eastman Dental Institute, University College London, London, United Kingdom*

Targeting with synthetic peptides is a promising approach in PDT for improving both the efficiency and selectivity of photosensitiser delivery. In particular, for ALA-PDT, the incorporation of ALA into a peptide prodrug offers a means to overcome well-known problems associated with the use of ALA itself, namely its lack of stability at physiological pH and its highly hydrophilic nature, that prevent it from crossing biological membranes and hence limits tissue penetration. In this work we report the synthesis and characterisation of a series of dipeptide prodrugs of general structure Ac-Xaa-ALA-OR, where Xaa is an alpha amino acid, chosen to provide a prodrug with appropriately tailored lipophilicity and water solubility. The uptake of the compounds and metabolism to PpIX in PAM212 keratinocytes, relative to ALA is evaluated by fluorescence spectroscopy, and further quantified by recovery and chemical derivatisation of intact/partially metabolised prodrugs. Based on these results, we attempt to rationalise efficiency of uptake in terms of calculated (i.e. clogP) and experimental HPLC-derived descriptors of hydrolipophilic balance for the compounds. The potential application of these findings for the synthesis of second generation ALA-peptides will also be discussed.

OC115

Histamine, nitric oxide and prostaglandin E2 are released following topical ALA-PDT of human skin. 'Real-time' investigation using intradermal microdialysis

Rebecca Brooke[1], *Meneka Sidhu*[1], *Rachel Watson*[1], *Martin Church*[2], *Peter Friedmann*[2], *Geraldine Clough*[2], *Lesley Rhodes*[1]
[1]*Photobiology Unit, Dermatological Sciences, University of Manchester, Manchester, United Kingdom;* [2]*Dermatopharmacology Unit, University of Southampton, Southampton, United Kingdom*

An inflammatory skin reaction is observed following topical 5 aminolevulinic acid photodynamic therapy (ALA PDT). We wished to examine the nature of the clinical response and to assess whether inflammatory mediators histamine, nitric acid and prostaglandin E_2 (PGE_2) may be involved, with direct sampling by cutaneous microdialysis.
0.25g/cm^2 of 20% ALA in ungentum merck (Porphrin, Crawford Pharmaceuticals, UK) was applied to a 10x 4cm area of the volar forearm skin of 7 healthy individuals. After 5h the site was irradiated with broadband red light (Waldmann 1200 L PDT, 600-750 nm, 100J/cm^2, 75mW/cm^2). The clinical response was noted and erythema was quantified at intervals to 24h using a reflectance instrument. Intradermal microdialysis fibres (5kD) were inserted and perfused with Ringers solution. Collections were made at 6 time points: pre-PDT, during PDT, and at 30 mins, 1h, 4h and 24h post-PDT. Microdialysate was snap frozen and stored at -70°C until analysis. Histamine and PGE_2 were measured by ELISA and nitric oxide by chemiluminescence assay. An immediate wheal and flare response was observed in all subjects, maximal at 30-40 minutes post PDT. This resolved to be followed by a prolonged erythema limited to the area of ALA application; the erythema index was significantly increased at 3h and remained elevated to 24h post PDT ($p<0.01$). Histamine levels significantly increased, from a baseline mean of 22 (SEM 6) pg/ml, to a peak of 188.3 (50.1) pg/ml at 30 min post-PDT ($p<0.001$), reducing to 77.9 (15.9) pg/ml at 4h, and returning to baseline by 24h with 11.5 (2.6) pg/ml. Nitric oxide levels were also elevated, increasing from a baseline of 0.28 (0.08) µM to a peak during irradiation of 1.45 (0.33) µM ($p<0.05$), and remained above baseline at 24h with 0.50 (0.1) µM. PGE_2 was also detected, with 163.5 (22.5) pg/ml at baseline, increasing to 470 (241) pg/ml at 1h post-PDT, and decreasing to 79.7 (28.2) pg/ml by 24h.
Hence 2 distinct inflammatory responses occur in human skin post-PDT, an immediate urticarial reaction, followed by a more prolonged erythema. While the time course of histamine release mirrors that of the urticarial reaction, PGE_2 and NO may also contribute to the acute phototoxic response. Pharmaceutical manipulation of these mediators may potentially influence the therapeutic response to ALA-PDT.
This project was funded by the British Skin Foundation.

OC116

Enhanced porphyrin accumulation using peptide derivatives of 5-aminolaevulinic acid for photodynamic therapy

Ludovic Bourre[1], *Francesca Giuntini*[2], *Ian M. Eggleston*[2], *Michael Wilson*[3], *Alexander J. MacRobert*[1]
[1]*National Medical Laser Center, University College of London, London, United Kingdom;* [2]*Department of Pharmacy and Pharmacology University of Bath, Bath, United Kingdom;* [3]*Division of Microbial Diseases, UCL Eastman Dental Institute, University College London, London, United Kingdom*

Intracellular porphyrin generation following administration of 5-aminolaevulinic acid (ALA) has been widely used in photodynamic therapy. However, ALA is hydrophilic which limits its bioavailability. We have developed more lipophilic ALA-peptide conjugates, which are uncharged at physiological pH yet still water soluble, for improving ALA delivery. In this study we investigated a phenylalanine conjugate with ALA (Ac-Phe-ALA-OMe).
Fluorescence pharmacokinetics of the induced protoporphyrin IX (PpIX) were studied in the transformed PAM212 keratinocyte cell line and pig skin explants. Photodynamic efficacy was assessed using the MTT assay. Then the influence of incubation temperature and the effect of peptide transporter inhibitors on PpIX synthesis were examined.
The intracellular porphyrin production was substantially increased with Ac-Phe-ALA-OME compared to equimolar ALA: after 5h of incubation with 0.1mM of prodrugs, the PpIX production was 308 and 61 ng/mg protein respectively for Ac-Phe-ALA-OMe and ALA. The phototoxicty study showed good correlation with the PpIX levels, with an LD_{50} at 25µM for ALA, 6µM for ALA-hexylester and 2.6µM for Ac-Phe-ALA-OMe. The PpIX fluorescence level was considerably reduced when cells were incubated with Ac-Phe-ALA-OMe at 4°C compared to 37°C, consistent with active transport. Studies using peptide transporter inhibitors showed that only cefadroxil caused a significant decrease in PpIX production with Ac-Phe-ALA-OMe. Finally, Ac-Phe-ALA-OMe produced higher porphyrin fluorescence in the skin explants than ALA by a factor of three.
These results show that the use of a suitable dipeptide prodrug of ALA can greatly increase its cellular uptake, generating more intracellular PpIX and improved tumour cell photosensitisation.

IL117

Polymorphic light eruption is associated with abnormalities in keratinocyte responses to UVR

Susan Lesley Walker
St John's Institute Dermatology, King's College London, London, UK

It is well established that the ultraviolet component of sunlight (UVR, ~295-400nm) suppresses cell-mediated immunity in normal healthy humans. It has been postulated that, in the short term, this immune suppression is an advantageous physiological response to UVR exposure, which prevents autoimmune responses to UVR-damaged skin. Patients with polymorphic light eruption (PLE) demonstrate a delayed-type hypersensitivity response to UVR, suggestive of an immune response to a UVR-induced antigen. Our previously published work, indicates that PLE patients have a flaw in the immunoregulatory response to UVR, which may contribute to the expression of their disease. In order to define the molecular events involved, we have established primary keratinocyte lines from 8 patients with severe PLE and 8 gender, age and skin-type matched controls. Preliminary data indicates that PLE keratinocytes release elevated levels of 3 soluble mediators that play an important role in Langerhans cell maturation and T-cell activation and recruitment, GM-CSF, RANTES and IP-10. We further characterised the abnormal response to UVR by extracting RNA from these cell-lines and quantifying gene expression using oligonucleotide microarrays and real-time PCR. By analysis of variance, 1979 genes were regulated by UVR. The effect of UVR on gene expression did not differ significantly between PLE patients and controls for any gene, but consideration of expression levels of all 32 samples (UVR and non-UVR exposed) showed that 16 genes were differentially expressed in PLE compared to controls. For eight of these 16 genes, we attempted to confirm the array results with real-time PCR, and we succeeded for six genes. Of the 16 genes, two have functions closely associated with the clearance of apoptotic cells: complement 1s subunit and scavenger receptor B1. Four others, namely fibronectin-1, immunoglobulin superfamily member-3, caspase-1 and paraoxonase-2 may also play a role in this process. The observation that these genes are involved in the clearance of apoptotic cells implicates this process in the pathogenesis of PLE. We postulate that a defect in the clearance of apoptotic cells, in combination with the presence of activated Langerhans cells may account for the failure to undergo immunosuppression and result in an autoimmune response to apoptotic debris.

IL118

Unbalanced immune reactions to UV-B irradiation in Polymorphic Light Eruption

A Soe Janssens, Stan Pavel, Frank R de Gruijl
Leiden Univ Med Ctr, Leiden, Netherlands

In a normal physiologic response to UV-B irradiation of the skin, both inflammation and down-regulation of cellular immunity occur when the UV dose exceeds certain thresholds which vary between individuals. Inadequate down regulation has been suspected to give rise to allergic reactions to the sun-exposed skin, as observed in Polymorphic Light Eruption (PLE). British groups provided experimental proof that PLE patients indeed show less immunosuppression at UV-B exposures around 1 MED (minimal erythemal dose) when compared to healthy controls. In earlier experiments we found that patients in which UV-B can provoke PLE show reduced migratory responses of Langerhans cells and neutrophils in the skin upon UV-B overexposure (6 MEDs). After mild UV-B hardening, these migratory responses after 6 MED appeared to normalize (MED increased by about 40% after hardening). In a follow-up clinical experiment we found no explanation for the reduced migratory responses in cytokine profiles (incl. IL1beta, TNFalpha, IL4, IL8, MIP-1alpha, -1beta and MCP1) in the fluid of blisters raised in skin of PLE patients after UV-B exposure. We did, however, find decreased IL1Ra/IL1alpha and IL1Ra/ IL1beta ratios in PLE patients, indicating a bias toward inflammatory reactions. UV-B hardening could perhaps be effective through correcting the inflammatory bias in PLE patients by increasing the erythemal threshold dose in relation to immunosuppressive threshold dose.

IL119

Regulation of murine asthma models by UV radiation of skin

Prue H Hart, Jacqueline P McGlade, Shelley Gorman, John J Finlay-Jones
Telethon Institute for Child Health Research and Centre for Child Health Research, University of WA, Perth, WA, Australia

Most investigations have shown a suppression by UVB radiation of Th1-driven immune responses. This study investigated the systemic effects of UVB on Th2-associated immune responses using two murine models of allergic respiratory inflammation, and the mechanisms of regulation involved. BALB/c mice were UVB irradiated (8 kJ/m2) three days before sensitization (and boost after 14 days) intraperitoneally with ovalbumin and the adjuvant, alum. After 21 days, airway-associated, asthma-like responses to aerosolized ovalbumin were analysed. Erythemal UVB irradiation of skin significantly suppressed airway hyperresponsiveness to methacholine and ovalbumin-specific responses in the lung-draining lymph nodes and in the lung compartment, including reduced inflammatory cells and cytokines in the bronchoalveolar lavage fluid. Further, reduced ovalbumin-specific responses by lung draining lymph node cells from mice that received 5 million lung draining lymph node cells from UVB-irradiated, but not from non-irradiated ovalbumin-sensitized mice suggested that UVB-induced regulatory cells were responsible for many of the asthma-reducing effects of dorsal UVB exposure. In a second model, C57BL/6 mice were irradiated with 4 kJ/m2 UVB before intranasal sensitization (and boost after 2 weeks) with papain, a cysteine protease homologue of the dust mite allergen Der p 1. No adjuvant was used. UVB irradiation reduced in vitro papain-specific responses by lung-draining lymph node cells harvested 24 hours after papain intranasal challenge two weeks after papain boost. The effects of UVB on this model of allergic respiratory inflammation were also measured in histamine receptor 1 -/- but not in histamine receptor 2 -/- mice, and implicate a role for the histamine type 2 receptor in UVB-induced suppression of antigen-specific responses in the draining lymph nodes. Thus, UVB-irradiation of skin can suppress airway hyperresponsiveness and cellular responses of the airways to respiratory allergens and implicates UV or its downstream mediators as a potential approach to reducing the severity of asthma.

IL120

The role of UV-induced chemokines in modulating immune responses

Scott N. Byrne[1], Alberto Y. Limon-Flores[2], Stephen E. Ullrich[2]
[1]Department of Dermatology, University of Sydney, Sydney, NSW, Australia; [2]Department of Immunology, UT MD Anderson Cancer Center, Houston, TX, USA

Chemokines (chemotactic cytokines) direct cellular traffic both to and from sites of inflammation. The release of chemokines following an inflammatory stimuli such as UV radiation can have profound effects on immunity. Considering the importance of cell movements following exposure to UV radiation (e.g. Langerhans Cells and Regulatory T cells) it is somewhat surprising that UV-induced chemokines have received almost no research attention. A lack of chemokine inhibitors and the limited availability of chemokine knockout animals may help explain this deficiency. Here we provide data indicating that UV regulates immunity by modulating chemokine-induced mast cell migration. Exposure to an immunosuppressive dose of UV radiation induced mast cell

accumulation in the skin-draining lymph nodes. Tissue and bone marrow derived mast cells express high levels of the CXCL12 chemokine receptor CXCR4, and UV exposure caused a significant upregulation of the chemokine CXCL12. Treating mice with a specific CXCR4 inhibitor, AMD3100 blocked mast cell migration and prevented UV-induced immune suppression. Thus, chemokine driven migration of dermal mast cells into lymph nodes is required for the induction of immune suppression and may serve as novel target for immunotherapy.

OC121

Chronotherapy - Circadian rhythm, the human immune response and treatment of disease

Joan Elizabeth Roberts

Fordham University, 113 West 60th Street, New York City, New York, United States

The immune system is susceptible to a variety of stresses. Recent work in neuroimmunology has begun to define how mood alteration, stress, the seasons, and daily rhythms can have a profound effect on immune response through hormonal modifications. Central to these factors may be light through an eye-brain hormonal modulation.

In adult primates, only visible light (400-700 nm) is received by the retina. When visible light excites melanopsin within specific retinal ganglion cells' the signal is directed through the optic nerve to the SCN, which controls a resultant circadian hormonal cascade which modulates the pituitary and pineal gland and eventually the thyroid and adrenal response. Melatonin, norepinephrine and acetylcholine decrease with light activation, while cortisol, serotonin, gaba and dopamine levels increase. These induced neuroendocrine changes can lead to alterations in mood and circadian rhythm as well as immune modulation. Several disorders, including cancer and autoimmune responses may be more effectively treated by matching the time of day [or the dark/light cycle] with a particular therapy.

OC122

UVB radiation and vitamin D influence the neonatal skin immune system and can have long term implications

Gregory M Woods, Roslyn C Malley, Heather M McGee, H Konrad Muller

Menzies Research Institute, University of Tasmania, Hobart, Tasmania, Australia

Exposure to environmental factors in early can life influence health and disease in adulthood. As the neonatal skin immune system has evolved to induce immunosuppression against commonly encountered antigens it prevents potentially damaging immune responses. However this immunosuppression could be disadvantageous as it could lead to a lack of anti tumour immunity if exposed to carcinogens in early life. One such carcinogen is UVB radiation, which is important for the production of vitamin D3 but excess exposure in childhood has been linked to skin cancer development in later life. We developed two mouse models to assess this dual effect of UVB radiation. The first model involves exposing neonatal mice to solar simulated radiation (ssUVR) as a source of UVB, and investigating the immediate and long-term effects on immune cells and responses. Immediately following ssUVR epidermal Langerhans cell density was reduced in a dose dependent manner compared to unirradiated mice, but there was no significant change in any lymph node or spleen cell populations. At 8 weeks of age, the LC network appeared normal, but lymph nodes from mice treated with ssUVR as neonates had an increase in the percentage of T regulatory cells (Tregs) and B cells. Thus, a single exposure of mice to ssUVR in the neonatal period has the ability to increase the proportion of these cells in adulthood, which may promote a suppressive environment. For the second model we developed a 25-hydroxyvitamin D3 (25(OH)D3) deficient population of BALB/c mice, through dietary vitamin D3 restriction. Adequate levels of circulating 25(OH)D3 significantly reduced the contact sensitivity response in adult male, but not female, mice. Analysis of neonatal tolerance revealed that adequate circulating 25(OH)D3 supported the induction of tolerance, more so in male than female mice, suggesting that the timing of 25(OH)D3 deficiency may determine the immunological outcome. These results indicate that vitamin D3 does play a role in regulating cutaneous immunity and the observation that neonatal, but not adult, female mice respond to the immunomodulatory effects of circulating 25(OH)D3 has implications for the skin immune system function and potential susceptibility to autoimmune conditions. The neonatal skin immune system is particularly sensitive to environmental influences such as UVB radiation and vitamin D3 deficiency, which can lead to implications in later life.

OC123

Cis-urocanic acid initiates gene transcription independently of the serotonin 5HT2A receptor in primary human keratinocytes

Kazuyo Kaneko[1], Ulli Smetana-Just[1], Mary Matsui[2], Antony R Young[1], Susan John[3], Mary Norval[4], Susan L Walker[1]

[1]St John's Institute of Dermatology, King's College London, London, UK; [2]Clinique Laboratories, Melville, NY, USA; [3]Immunobiology, King's College London, London, UK; [4]Medical Microbiology, University of Edinburgh, Edinburgh, UK

Urocanic acid (UCA) is a major epidermal chromophore that undergoes *trans* to *cis* photoisomerisation following exposure to solar ultraviolet radiation (UVR). Although there is considerable evidence that *cis*-UCA suppresses cell-mediated immune response in mouse skin, the molecular events are not fully understood. Recent evidence from studies in mice suggests that the serotonin $5HT_{2A}$ receptor responds to *cis*-UCA. However, whether the $5HT_{2A}$ receptor is the functional *cis*-UCA receptor in humans remains controversial. In this study, we examined involvement of gene transcription and the $5HT_{2A}$ receptor in the immunomodulatory effects of *cis*-UCA on primary human keratinocytes.

Primary human keratinocytes were treated with *trans*- or *cis*-UCA (10-100μg/ml), serotonin (0.1-1mM), solar simulated UVR (12J/cm^2 ~2-3 minimal erythema doses for fair skin) or PBS. After 24hrs, total RNA and cell culture supernatant were prepared for the gene array, TaqMan real-time RT-PCR and ELISA analyses.

The gene array results showed that about 400 genes were induced by UVR, 16 of which also up-regulated by c*is*-UCA. In contrast, *trans*-UCA had no effect on gene expression. The genes up-regulated by both *cis*-UCA and UVR were associated with apoptosis, cell growth arrest, cytokines and oxidative stress. Selected genes were also quantified independently using real time RT-PCR to confirm the array results (median increase of 4.2 fold at *cis*-UCA 10μg/ml). Prostaglandin-endoperoxide synthase 2 was dramatically induced by *cis*-UCA, resulting in an enhanced secretion of prostaglandin E_2 (PGE_2) into the cell culture supernatant. UVR had the same effect, but *trans*-UCA had no effect on PGE_2 production. Unexpectedly, the $5HT_{2A}$ receptor was not detected in primary human keratinocytes (n=11) though it was expressed in primary human fibroblasts. In addition, unlike *cis*-UCA, serotonin did not alter the cell growth, cell death or PGE_2 synthesis, suggesting that the $5HT_{2A}$ receptor is unlikely to be the functional receptor for *cis*-UCA in primary human keratinocytes.

OC124

Modulation of photoimmune suppression by exogenous or endogenous melatonin

Kishen Lachireddy, Diane Domanski, Vivienne E Reeve

University of Sydney, Sydney, Australia

In humans and mice, a major route for the initiation of UV radiation-induced immunosuppression is via the photoisomerisation of naturally occurring epidermal *trans*-urocanic acid to the *cis*

isomer. *Cis*-urocanic acid activates peripheral sensory nerves, stimulating the release of immunosuppressive neuropeptides such as calcitonin gene related peptide (CGRP) and substance P, thus involving the neuroendocrine system in the establishment of photoimmune suppression. The receptor for *cis*-urocanic acid has remained elusive until the recent demonstration that *cis*-urocanic acid binds competitively to the 5-hydroxytryptamine (5-HT, serotonin) receptor, and acting as a 5-HT agonist, causes the release of inflammatory and immunosuppressive mediators from T lymphocytes. We have examined the potential of the pineal neurohormone, melatonin (N-acetyl-5-methoxytryptamine), to antagonise *cis*-urocanic acid, as its presence in cells is negatively correlated with 5-HT (the substrate for its synthesis) and it has been reported to regulate 5-HT receptor binding via a feedback loop. Melatonin is best recognised for regulating circadian rhythms, and is synthesised during the dark photoperiod largely by the pineal gland, but it also has antioxidant, anti-cancer and immune protective or restorative functions described in various disease models.

The majority of laboratory mouse strains have been found to have extremely low or undetectable levels of melatonin, with the exception of the CBA and related C3H strains. We have examined the photoprotective effects of melatonin in both the C3H and the Skh:hr-1 hairless mouse, which differ in measurable pineal and plasma melatonin level, but which we found respond similarly to melatonin-modifying treatments.

Melatonin was fed in the drinking water (31.25 – 125 ug/day) and dose-dependently protected against the suppression of contact hypersensitivity by solar simulated UV irradiation (SSUV). When endogenous melatonin in C3H mice was inhibited by treatment with the beta-adrenergic receptor antagonist propanolol, SSUV-suppression of contact hypersensitivity was exacerbated. Similarly, when 24-hour dark cycle was employed to inhibit the endogenous synthesis of melatonin, mice showed greater immune suppression by both SSUV and *cis*-urocanic acid.

It is interesting that melatonin has been linked with oestrogen activity, and with antioxidant activity (stimulated GSH pathways, protected haem oxygenase, correlated with metallothionein induction), functions known to be photoimmunoprotective. Future studies aim to understand the links between these factors and to elucidate the downstream immune-determining pathways.

OC125

Upregulation of pro- and anti-inflammatory lipid mediators during the sunburn response in human skin. Candidates for mediation and regulation of UVR-induced neutrophil chemotaxis

A. Haylett[1], M. Masoodi[2], M. Brownrigg[1], K. Gledhill[3], D.J. Tobin[3], A.J. Thody[4], A. Nicolaou[2], L.E. Rhodes[1]

[1]Photobiology Unit, Dermatological Sciences, University of Manchester, Manchester, UK; [2]Pharmaceutical Sciences, University of Bradford, Bradford, UK; [3]Biomedical Sciences, University of Bradford, Bradford, UK; [4]School of Clinical and Laboratory Sciences, University of Newcastle, Newcastle, UK

Sunburn is an acute inflammatory process, with vasodilatation and leucocyte chemotaxis occurring as central features. Fatty acid derived mediators are believed to play pivotal roles in this process, following their metabolism by cycloxygenases (COX), lipoxygenases (LOX) and CYP450 subsequent to UV-induced hydrolysis of membrane lipids. We therefore examined for the presence of pro-inflammatory and anti-inflammatory eicosanoids formed during the course of the sunburn response in human skin.

22 healthy humans (9M: 13F, mean age 40 yrs, range 28– 52 yrs, skin type I-IV) were irradiated with 4 MED of UVR (UV6 lamps, 290-400 nm) on buttock skin and the erythema quantified over 72h using a reflectance instrument. Suction blisters were raised at 4, 18, 24, 48 and 72h post-UVR, and from unirradiated skin. Suction blister fluid was aspirated and snap-frozen at -70°C prior to analysis using electrospray liquid chromatography tandem mass spectrometry (ESI-LC-MS-MS). Data were analysed by ANOVA. The erythema index peaked at 24h post-UV and was maintained to 72h, $p<0.001$. Omega-6 polyunsaturated fatty acid (PUFA) derived vasodilatory prostaglandins PGE_2 and PGF_{2a} were significantly raised in the first 24 and 18h, respectively, $p<0.05$, while the n-3 PUFA derived partial agonist PGE_3 was significantly raised to 48h, $p<0.05$. The potent neutrophil chemotactin 12-monohydroxy-eicosatetraenoic acid (-HETE), derived through 12-LOX, showed significant elevation at all time points from 18-72h, $p<0.0001$. Similarly neutrophil chemotactin 8-HETE was elevated at 24-72h post UVR ($p<0.01$), while the neutrophil chemotactin 11-HETE was significantly raised post UVR from 4h onwards ($p<0.001$). In contrast, neutrophil chemotactin leukotriene B4 (LTB_4) was not detected. The potent anti-inflammatory 15-LOX derived mediator 15-HETE, reported to inhibit synthesis of 12-HETE and LTB_4, was significantly increased at 24-72h, peaking at 72h, $p<0.01$.

Hence, the combined influence of a series of UVR-induced prostaglandins may contribute to vasodilatation during the sunburn response. Our data supports the monohydroxy-eicosatetraenoic acids 12-HETE, 11-HETE and 8-HETE, rather than LTB_4, as candidate mediators of UVR-induced neutrophil chemotaxis, while the anti-inflammatory metabolite 15-HETE may provide regulation of the inflammation.

This research was funded by the Wellcome Trust (grant no. WT077714).

OC126

A characterisation of iNKT cells in mouse skin

Kirsten J.L. Hammond, Carling Y-Y. Chan, Gary M. Halliday

Dept. Dermatology, University of Sydney, Sydney, NSW, Australia

'Invariant' (*i*) NKT cells are an evolutionarily conserved T cell subset that use a single T cell receptor (TCR) alpha chain (Valpha14Jalpha18 in mice; Valpha24Jalpha18 in humans) combined with a limited repertoire of TCR alpha chains. The highly restricted TCR repertoire of *i*NKT cells recognises glycolipid antigens presented by the antigen-presenting molecule CD1d, which immediately distinguishes them from most other alphabeta T cells that recognise peptides in conjunction with major histocompatibility complex (MHC) class I or II molecules. *i*NKT cells have the ability to powerfully influence immune responses due to their capacity to rapidly produce copious amounts of immuno-modulatory cytokines.

Here we report for the first time the direct identification of *i*NKT cells isolated from normal mouse skin. We used a highly specific reagent, alphaGalCer-loaded CD1d tetramers, and flow cytometry to detect these cells. This observation has important implications for skin immunity – for example, a glycolipid extracted from Borrelia burgdorferi, the causative agent of Lyme disease which infects humans through deer tick bites, has recently been found to stimulate *i*NKT cells. In addition, *i*NKT cells have been implicated in the mechanism by which ultraviolet (UV) radiation from sunlight suppresses the immune system – a significant contributing factor in the development of skin cancer induced by UV radiation. Our novel findings, that *i*NKT cells are present in skin in large numbers, indicate that these cells may represent major mediators of skin immunity.

IL127

TCSPC fluorescence lifetime imaging in photobiology

Wolfgang Becker, Axel Bergmann

Becker & Hickl GmbH, Berlin, Germany

Multi-dimensional time-correlated single photon counting (TCSPC) is based on the excitation of the sample by a high-repetition rate pulsed laser and the detection of single photons of the fluorescence signal in several detection channels. Each photon is characterised by its time in the laser period, its detection channel number, and the

coordinates in the scanning area. The recording process builds up a multi-dimensional photon distribution over these parameters. Combined with a confocal or two-photon laser scanning microscope, multi-dimensional TCSPC makes a fluorescence lifetime imaging (FLIM) technique with multi-wavelength capability, near-ideal counting efficiency, picosecond time resolution, and the capability to resolve multi-exponential decay profiles. We will show that TCSPC FLIM in fact adds four new dimensions to multi-dimensional microscopy. The technique is particularly useful for autofluorescence imaging of tissue and fluorescence resonance energy transfer (FRET) experiments in cells. We will demonstrate the capabilities of the technique for a number of typical applications.

IL128

New Total Internal Reflection (TIR) techniques for live cell imaging

Herbert Schneckenburger[1], Michael Wagner[1], Petra Weber[1], Thomas Bruns[1], Brigitte Angres[2], Heiko Steuer[2], Wolfgang S.L. Strauss[3]

[1]Hochschule Aalen, Institut für Angewandte Forschung, Anton-Huber-Str. 21, 73430 Aalen, Germany; [2]NMI Naturwissenschaftliches und Medizinisches Institut an der Universität Tübingen, Markwiesenstr. 55, 72770 Reutlingen, Germany; [3]Institut für Lasertechnologien in der Medizin und Messtechnik an der Universität Ulm, Helmholtzstr. 12, 89081 Ulm, Germany

For more than 20 years total internal reflection (TIR) of light has been used to study cell-substrate surfaces and to get some detailed informations of cell membranes. When a light beam propagating through a medium of refractive index n_1 (e.g. glass) meets an interface with a second medium of refractive index $n_2 < n_1$ (e.g. cytoplasm), total internal reflection occurs at all angles of incidence Θ which are greater than a critical angle Θ_c. Despite being totally reflected, the incident beam establishes an evanescent electromagnetic field that penetrates into the second medium and decays exponentially with the distance z from the interface. Therefore, fluorophores located within or close to the plasma membrane are detected selectively in living cells.

Presently 3 different devices for total internal reflection fluorescence microscopy (TIRFM) are reported. In the first case the illumination angle and thus the penetration depth of the evanescent electromagnetic field can be varied between about 70 nm and 300 nm, and cell-substrate topology can be determined with nanometre precision. This technique has been used to study cell adhesion upon photodynamic therapy (PDT). A second device is based on focusing of a laser beam to a small area in order to detect focal adhesions or protein-protein interactions in plasma membranes which play major roles in cell adhesion, intracellular signalling or pathogenesis of various diseases. In both devices cells are connected to a glass prism, whereas in a third device a high aperture microscope objective lens is used for TIR illumination. This last device has already been commercialized and offers the advantage of low background luminescence (needed e.g. for Raman microscopy), but is rather unflexible concerning microscope lenses, object fields and illumination angles.

Based on the microscopic results a fluorescence reader for standardized samples (e.g. confluent cells in microtiter plates) was developed. Here, 96 samples are excited simultaneously by TIR in view of future diagnostic and pharmaceutical applications.

IL129

Time-resolved fluorescence imaging in the life sciences

Klaus Suhling

Department of Physics, King's College London, London, UK

Fluorescence imaging techniques are powerful tools in the biological and biomedical sciences, because they are minimally invasive and can be applied to live cells and tissues. The fluorescence emission can be characterised not only by its intensity and position, by also by its fluorescence lifetime, polarization and wavelength. Fluorescence Lifetime Imaging (FLIM) in particular has emerged as a key technique to image the environment and interaction of specific proteins in living cells. GFP-labelled proteins in cells were imaged using a time-correlated single photon counting (TCSPC)-based confocal scanning FLIM set-up. In addition, the potential of time-resolved wide-field photon counting imaging based on a photon counting image intensifier will be discussed.

http://kcl.ac.uk/schools/pse/physics/people/klaussuhling.html

IL130

Multiwavelength fluorescence lifetime imaging (SLIM) in photobiology

Angelika Rueck[1], Frank Dolp[1], Björn v. Einem[2], Cornelia Steinmetz[2], Christine v. Arnim[2], Rudolf Steiner[1]

[1]ILM, Helmholtzstrasse 12, 89081 Ulm, Germany; [2]Neurology, University Ulm, Ulm, Germany

SLIM (spectral fluorescence lifetime imaging) is a highly sophisticated new technique, which combines spectral resolved and time resolved detection[1]. Real spectral information is achieved by using a grating in front of a PML-array, which allows time-correlated single photon counting (TCSPC). Whereas spectrally resolved fluorescence imaging alone has a reasonable sensitivity, the specificity of fluorescence detection can be improved by considering the fluorescence lifetime.

SLIM was realized on the basis of a laser scanning microscope (LSM410, Zeiss, Germany). The fluorescence light from the second descanned detection channel was coupled into a 600 µm multimode fibre. The end of the fibre was put into the input focal plane of an MS125 spectrograph (grating of 600 lines/mm, LOT-Oriel). A PML-16 multichannel PMT module (Becker&Hickl GmbH, Berlin, Germany), containing a 16 channel Hamamatsu R5900-01-L16 multi-anode PMT and the TCSPC routing electronics was attached to the output of the spectrograph. The grating yields a 200 nm spectral range spread over the 16 channels of the detector. The spectral bandwith of the PMT channels was about 12 nm. For fluorescence excitation, a Ti:Sa laser or alternatively a ps diode laser was used.

The various possibilities which SLIM offers in photobiology, for example to improve cell diagnosis will be discussed as well as successfully realized applications. These include cancer diagnosis, and FRET measurements for multiple protein interactions, related to various diseases[2]. Pitfalls due to photobleaching and scattering under various excitation conditions will be discussed as well.

[1]A. Rück, C.H. Hülshoff, I. Kinzler, W. Becker, R. Steiner. SLIM: A new method for molecular imaging. Micr. Res. Tech., 70, 485-492 (2007). [2]A. Rück, F. Dolp, R. Steiner, S. Lintner, B. v. Einem, C. v. Arnim. SLIM: A sophisticated method for molecular imaging. SPIE Proceedings, Photonics West, 6442, 64420Y-1 (2007).

IL131

Multiphoton tomography in medicine using femtosecond lasers

Karsten König[1], Iris Riemann[1], Frank Stracke[1], Aisada Uchugonova[1], Rainer Bückle[2], Ronan Le Harzic[2], Katja Schenke-Layland[3], Martin Kaatz[4], Peter Elsner[4]

[1]Fraunhofer Institute for Biomedical Technology, St. Ingbert, Germany; [2]JenLab GmbH, Jena, Germany; [3]Cardiovascular Research Laboratory, University of California, Los Angeles, USA; [4]Department of Dermatology, Friedrich-Schiller University Jena, Jena, Germany

Near-infrared femtosecond laser pulses have been used to realize non-invasively high-resolution 5D imaging of intra-tissue cells and extra-cellular matrix components. We report on the clinical use of the multiphoton tomograph DermaInspect as well as a rigid Grin lense mini-endoscope. These novel laser systems have been used to

image disorders of human skin and cardiovascular and ocular tissues. In particular, patients with melanoma have been analyzed. Melanocytes, macrophages as well as elastin and collagen network could be detected and imaged with submicron spatial resolution, 50 picosecond temporal resolution and 10 nm spectral resolution. So far, multiphoton tomography has been performed for cancer diagnosis and in situ drug screening on more than 500 patients.

OC132

Measurement and assessment of circadian effective radiation of natural and artificial sources

Dieter Kockott[1], *Helmut Piazena*[2], *Rüdiger Sippel*[3]

[1]DK UV-Technik, Hanau, Germany; [2]Klinik für Psychiatrie der Charite, Berlin, Germany; [3]MSS Elektronik GmbH, Fröndenberg, Germany

Many biological processes are controlled by optical radiation. Only recently, the responsible circadian photo sensors on the retina have been discovered, and their action spectrum has been measured. By using these experimental data, Gall and Lapuente defined the circadian action spectrum c(λ).

The integral measuring radiometer MSS 1000 uses a receiver whose spectral sensitivity $s_{rec}(\lambda)$ is well adjusted to the action spectrum c(λ). It measures the circadian effective irradiance E_{circ} directly. The contribution includes technical details like spectral sensitivity, cosine response, and comparison to results obtained by using a high sophisticated spectral radiometer equipped with double monochromator. We got a linear relationship between the circadian effective irradiances when measured by both MSS 1000 and the spectral radiometer. The linearity covers 4 orders of magnitude even when using a variety of light sources including solar radiation, incandescent lamps, halogen lamps, and different kinds of fluorescent lamps.

Due to the fact that the specific human receivers for circadian effective radiation are located in the retina of the eye, the circadian effective irradiance ought to be measured at the retina, which is impossible. However, it is possible to consider the transmittance of the eye lens being age-dependent by calculation. This personal related assessment of the measured circadian effective irradiance is automatically taken into account by the instrument. Results depending on age are presented.

The new instrument MSS 1000 is useful, among others, for measuring threshold values for circadian effective irradiance and radiant exposure (dose) depending on the age of the person considered, or for monitoring the circadian effective exposure of people at workplaces.

OC133

Instrumentation for selective fluorescence detection from deeper layers of the tissue

Jonas Venius, *Ricardas Rotomskis*

Vilnius University, Physics Faculty, Laser Research Center, Vilnius, Lithuania

In conventional optical biopsy the detected signal is a mixture of fluorescence signals originated in various depths. Most of the signal is composed from fluorescence of the upper layer and sometimes it drowns the useful information coming from the tissue inside. Signal from the deeper layers is weak due to the light scattering, but scattering also allows photons, originated deeper in the tissue, leave the medium at higher distances from the point of light incidence into the tissue, whereas photons, originated in the superficial layers, will escape the medium at smaller distances. Therefore, the distribution of fluorescence intensity registered on the tissue surface at different registration distances contains information about the depth dependent distribution of the fluorophores in the tissue.

The experiments, performed in the simulated turbid medium, demonstrated, that the special multidistance fiber optic probe used for fluorescence measurements, could be employed for fluorescence detection exclusively from the deeper or superficial layers. Detection fibers, situated near the illumination (excitation) fiber, collected more photons originated in the superficial layer, while detection fibers, located at higher distances appeared to be more sensitive to the deeper layers. However, the estimation of the exact depth from which the detected photon was emitted is still problematic due to the inhomogeneous properties of the tissue.

OC134

Real-time evaluation of tissue properties for feed-back dosimetry in interstitial photodynamic therapy

Johan Axelsson[1], *Ann Johansson*[1], *Johannes Swartling*[2], *Sara Pålsson*[2], *Thomas Johansson*[2], *Niels Bendsoe*[3], *Katarina Svanberg*[4], *Sune Svanberg*[1], *Stefan Andersson-Engels*[1]

[1]Department of Physics, Lund University, Lund, Sweden; [2]Spectracure AB, Lund, Sweden; [3]Department of Dermatology, Lund University Hospital, Lund, Sweden; [4]Department of Oncology, Lund University Hospital, Lund, Sweden

Prostate cancer treatment utilizing photodynamic therapy (PDT) has been reported to induce tissue necrosis and decrease in prostate specific antigen. The treatment response show large variations possibly due to biological variations. Our group is developing an instrument for interstitial PDT capable of delivering light into the prostate. The system utilizes real-time treatment feedback which relies on light transmission measurements conducted during the treatment session. The prostate geometry is imaged using ultrasound which renders a three-dimensional representation of the target volume. The optical fibers are then positioned using a iterative random-search algorithm to ascertain that the whole prostate can be treated. Before the treatment starts an optimization algorithm is run to predict individual fiber irradiation times. During the treatment the light irradiation halts during predefined time-intervals and the light transmission measurements are performed. The system can measure the treatment light transmission, NIR-light transmission and photosensitizer fluorescence. The measurements are then used to assess the effective attenuation coefficient, by means of spatially resolved spectroscopy, for the treatment light which forms the input to the optimization algorithm. Hence, the irradiation times for individual fibers are updated throughout the treatment in order to compensate for the influence of changes in tissue composition on the light distribution at the therapeutic wavelength. The presentation will focus on the dosimetry models developed for this novel instrument, allowing real-time treatment feedback.

PL135

Multiple regulatory pathways for photoimmune responsiveness

Vivienne E Reeve

University of Sydney, Sydney, NSW, Australia

The majority of the cutaneous impairments induced by UV radiation that lead to the suppression of T cell mediated immunity in humans and mice, have been assigned as products of the UVB waveband. These include the primary lesions, DNA damage, *cis*-urocanic acid formation, and cell membrane-derived oxidised lipid products, but there is also a deficit of functional Langerhans cells, and influx of mast cells, and an imbalance of epidermal cytokine expression, such that Th2-associated IL-4 and IL-12 are overexpressed at the expense of Th1-associated IL-12 and IFN-gamma. In contrast to UVB, environmentally relevant doses of UVA appear not to be immunosuppressive, but rather to moderate the immunosuppressive actions of UVB via the induction of the antioxidant stress enzyme haem oxygenase-1 in the skin, and to protect against the UVB alteration of the cytokine array, the induction of epidermal apoptosis, and the photocarcinogenic severity in mice.

Strategies to reduce photoimmune suppression have been found also to reduce photocarcinogenesis, for example treatment of mice with various antioxidant phytochemicals. The isoflavone equol has been of interest in determining whether its protective mechanism originates from its innate antioxidant radical scavenging capacity or its phytoestrogenic effects. Acting as an antioxidant, equol treatment enhanced the inducibility of haem oxygenase activity in mouse skin, and its photoimmune protection was reduced if haem oxygenase activity was inhibited. In addition, oestrogen receptor (ER) blockade in the mouse effectively and unexpectedly not only prevented photoimmune protection by equol, but alone also exacerbated photoimmune suppression, revealing a natural UV-protective role for ER signalling in the skin. This suggested a link between cutaneous immune regulatory antioxidant responses and the ER.

Recently we have identified ER-beta expression in mouse skin. In the ER-beta-/- mouse, we find an exaggerated UV-suppression of CHS, and an exacerbation of the epidermal cytokine disruption by UVB. It is consistent that human epidemiological studies have reported higher skin cancer incidences in females than in males, therefore our current studies are examining the susceptibility of this mouse to photocarcinogenesis. Furthermore, the ER-beta-/- mouse does not respond to the protection by UVA, consistent with defective antioxidant capacity. Future studies will clarify the relationship of the protective HO-1 response to ER-beta activity.

IL136

PDT effects on the tumor microenvironment: growing evidence for combined modality approaches

Charles J. Gomer

University of Southern California, Childrens Hospital Los Angeles, Los Angeles, CA, United States

Photodynamic therapy (PDT) induces significant changes within the tumor microenvironment that can lead to an angiogenic and/or a survival phenotype. We have previously reported that PDT can increase the expression of vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), and cyclooxygenase-2 (COX-2) within treated tumors. We have also observed that the expression levels of hypoxia inducible factor-1 (HIF-1), VEGF and MMP-9 within PDT treated tumors are proportional to the dose rate of delivered light. We recently discovered that survivin, a member of the inhibitor of apoptosis (IAP) family, is increased and activated in tumor cells and tissues following PDT. This presentation will address strategies on how best to combine PDT with clinically relevant anti-angiogenic therapy. The effectiveness of agents targeting different components of the VEGF signaling pathway (i.e. growth factor, receptor, and/or tyrosine kinase activity) will be presented. The possible role of VEGF in modulating tumor response following PDT delivered at low and high light dose rates also will be discussed. Finally, results will be presented from experiments designed to determine if PDT efficacy is altered by treatment-mediated activation of survivin.

IL137

Molecular response based combinations with PDT

T. Hasan, N. Solban, S.K. Chang, I. Rizvi, T. Stepinac, A. Liang, Z. Mai, H. Athar

Wellman Laboratories of Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

Photodynamic therapy (PDT) is now a reasonably well-known therapeutic option and is approved as a first line treatment of age-related macular degeneration (AMD), a non- oncologic condition. For most cancer applications PDT is still a palliative or adjunctive treatment often when all other options have failed. As the modality evolves toward becoming a first-line or curative option, long-term effects of processes involved will need to be studied. Cellular and tissue responses to PDT are more complex than responses to the more conventional therapies, perhaps because PDT is inherently a binary (or ternary) therapy. In addition to the nature and localization of the photosensitizer (PS), the timing of illumination after administration, the mode of administration and the PS and light doses, the efficacy and selectivity of responses are also determined by the physiology and geometry of tumors, the inherent survivability of tumor cells (in circulation and other anatomic sites) and cellular and molecular responses to PDT. A sound understanding of these factors offers an opportunity of designing intelligent mechanism-based combination treatment regimens that could result in better treatment outcome. This presentation will discuss of initial studies along these lines using a variety of mechanistic and imaging tools and implications of the results obtained.

IL138

Molecular effectors and modulators of cell death induced by PDT

Michael Dewaele[1], Esther Buytaert[1], Sofie Van Kelst[1], Wim Martinet[2], Jean-Yves Matroule[3], Jacques Piette[3], Patrizia Agostinis[1]

[1]Catholic University of Leuven, Leuven, Belgium; [2]University of Antwerp, Antwerp, Belgium; [3]University of Liege, Liege, Belgium

Hypericin-based photodynamic therapy (PDT) will be soon exploited as a new paradigm of anticancer therapy for the management of superficial bladder cancer. A better understanding of the molecular mechanisms underlying hypericin-mediated photokilling is therefore a pressing need to maximize its therapeutic benefit in clinical practice.

We recently described that photodamage to the sarco(endo)plasmic Ca^{2+}-ATPase (SERCA) pump and the subsequent loss of Ca^{2+} homeostasis, due to the selective accumulation of the hypericin in the ER, is the most apical molecular event causative for cell death[1]. Downstream of the ER-Ca^{2+} emptying, both caspase-dependent and -independent pathways are activated to ensure cell demise. The induction of apoptosis as a cell death modality is dependent on the availability of pro-apopototic Bax and Bak proteins, which are essential effectors of the mitochondrial membrane permeabilization and subsequent caspase activation[1]. Although previous investigation has shown cellular demise in murine embryonic fibroblasts (MEFs) doubly deficient for Bax/Bak (DKO) to be due to the induction of an autophagic cell death pathway[1], the exact role of autophagy in both apoptosis-competent or in apoptosis–deficient cells following hypericin-photosensitization is not completely understood. We are studying the signalling events underlying cell death in wild type cells and in cells in which mitochondrial apoptosis has been genetically knocked out. We are also addressing the role of autophagy in apoptosis-competent cells in which this catabolic process can be turned off by the conditional knock-out of the essential autophagy gene Atg5. Additionally, to shed more light into the molecular players of the cell death pathways in photosensitized cancer cells, we undertook a gene expression profiling analysis in bladder cancer cell lines[2]. This study reveals the coordinated induction of several genes involved in the unfolded protein response after PDT-induced ER stress and identifies in the activation of p38 MAPK a major signaling pathway required for the expression of key transcripts with an established tumor promoting role[2]. All together these recent studies indicate that ER-stress evoked by hypericin-mediated photodamage propagates cell death as well as survival pathways, which appear to share proximal molecular effectors. The identification of the molecular targets tipping the balance towards cancer cell death, both apoptotic and non-apoptotic, following hypericin-mediated PDT and their potential therapeutic value *in vivo* warrants further investigation.

[1]Buytaert et al, (2006) FASEB J. 20:756-8. [2]Buytaert et al, (2007) submitted.

IL139

NF-kB is a central element in the fate of cancer cells treated by photodynamic therapy

Jacques G Piette, Cédric Volanti, Sébastien Bontems, Jean-Yves Matroule

GIGA-Research, University of Liège, Liège, B4000, Belgium

Photodynamic therapy (PDT) is an established anticancer modality utilizing the photogeneration of reactive oxygen species (ROS) to kill the cancer cells. Unfortunately some cancer cells can survive to the treatment and regrowth causing tumor recurrencies. We were involved in the understanding of the molecular events that are induced in PDT-treated cancer cells. Among the pro-survival factors activated by PDT, one can cite several anti-apoptotic proteins such as the IAPs, XIAP and survivin together with the products of the cyclooxygenase-2 (COX-2) activity. The common denominator between all these proteins is the transcription factor NF-κB which is induced in cencer cells following PDT. NF-κB is a dimeric transcription factor kept inactive in the cytoplasm of untreated cells, and translocating into the nucleus of treated cells. This translocation is linked to the phosphorylation-degradation of its cytoplasmic inhibitor IκBα. PDT is known to activate the kinase complex which is phosphorylating IκBα leading to its degradation via the 26S proteasome. On the other hand, NF-κB is also controlling genes involved in neutrophils recruitment after PDT. Indeed, various cytokines such as TNF, IL-1 and IL-6 but also chemokines such as IL-8, MCP-1 and RANTES are controlled by NF-κB. In endothelial cells, adhesion molecules expression also depend upon NF-κB for their expression. All this means that the inflammatory branch of PDT can be tighly controlled by NF-κB activation. These data reveals the dual function of NF-κB in PDT-treated cancer cells because when activated it can protect cells from apoptosis but is also important for the inflammatory reaction induced by PDT.

OC140

The effects of iron on protoporphyrin IX accumulation, cellular damage (DNA damage) and cellular viability in aminolaevulinic acid and methyl aminolaevulinate photodynamic therapy

Andrew Pye, Alison Curnow

Cornwall Dermatology Research, Peninsula Medical School., Truro, Cornwall, United Kingdom

Photodynamic therapy (PDT) with aminolaevulinic acid (ALA) or its methyl ester (MAL) combines the selective accumulation of the photosensitiser protoporphyrin IX (PpIX) in tumour tissue with visible light (and tissue oxygen) to produce reactive oxygen species (ROS). The use of iron chelators in combination with ALA or MAL has been shown to increase the accumulation of PpIX in the cell by reducing its bioconversion to haem. Binding iron in this way should therefore increase the level of photosensitiser in the cell and subsequently increase the level of cellular damage mediated after light irradiation. Binding iron however may also have other effects on the cell as iron is an important micronutrient. In particular binding iron may affect the production of ROS through redox cycling.

The effects of iron in ALA-PDT or MAL-PDT were investigated in human cultured cells. Levels of PpIX accumulating in the cells after ALA or MAL administration were determined fluorometrically. Cells were then irradiated with red light (15 J/cm^2) or sham irradiated. Post irradiation the level of PpIX in the cells was assessed again. Cellular damage and cellular viability were then determined using single cell gel electrophoresis and trypan blue exclusion respectively. This protocol was then repeated with either iron sulfate, the iron chelator desferrioxamine (DFO) or the novel hydroxypyridinone iron chelator CP94, co-incubated with either ALA or MAL.

Increasing ALA or MAL concentrations led to the accumulation of greater levels of PpIX in the cells. These levels of PpIX were significantly reduced by the application of light. This was closely associated with the detection of increased levels of cellular damage and reduced cellular viability. No significant changes in PpIX, cellular damage or viability were detected in the un-irradiated cells. Co-incubation with the iron chelators resulted in increased levels of PpIX accumulating in the cells. These were translated into greater levels of cellular damage upon light irradiation. The opposite effect was observed in the iron + ALA co-incubated cells, with lower PpIX levels accumulating and lower levels of cellular damage detected. This study highlights the role of iron in ALA/MAL-PDT and adds to the growing evidence supporting the inclusion of iron chelators in ALA/MAL-PDT protocols to increase photosensitisation/clinical efficacy.

OC141

Intracellular and intratissular distribution of Foscan modulates apoptosis induced by photodynamic therapy

Sophie Marchal[1], Aurélie François[1], Julie Garrier[1], Aude Bressenot[2], François Guillemin[1], Lina Bezdetnaya[1]

[1]Nancy University, CNRS UMR 7039, Centre Alexis Vautrin, Nancy, France; [2]Nancy University, Department of Pathology, CHU Brabois, Nancy, France

Mechanisms of cell death triggered by photooxidatve stress are tightly related to the sites of photosensitizer accumulation. In this communication, we report the impact of intracellular or intratissular localisation of Foscan® on apoptosis induction following PDT.

In vitro, the relationship between the subcellular localisation of Foscan® and intrinsic apoptotic pathway post Foscan®-PDT was investigated in the human mammary adenocarcinoma MCF-7 cell line. Apoptosis was analysed through mitochondria response (release of cytochrome c and membrane depolarisation) and activation of post mitochondrial caspase cascade, caspases -9 -7 (MCF-7 cells lack caspase-3) and -6. Short time (3h) of incubation with Foscan® revealed localisation mainly in Golgi apparatus and endoplasmic reticulum (ER). After photosensitization immediate mitochondria photodamage was observed followed by a mild activation of caspases. Increase in incubation time from 3h to 24 h with Foscan® showed a progressive leakage of Foscan® from Golgi apparatus. Cells incubated 24 hours with Foscan® and subjected to equitoxic light doses yielded fluence-dependent enhanced induction of the ER-resident glucose related protein 78 (Bip/GRP78) underlining the ER as the main site of photodamage. The analysis of apoptotic molecular events revealed a decrease in mitochondria response whereas caspase-7 activation was increased and strongly related to the expression of GRP78. Taken together, these results demonstrate that Foscan® localisation in ER improves the activation of a caspase-7 dependent pathway suggesting that apoptosis can be favoured by modulating the duration of photosensitiser contact with the tissue. In this context, *in vivo* apoptosis was evaluated in human colon adenocarcinoma HT29 xenografted tumour in nude mice subjected to Foscan® injected at different time (3h, 6h and 24h drug-light interval, DLI) before irradiation (30 mW cm^{-2}, 10 J cm^{-2}). Apoptosis was investigated in tumour sections by immunohistochemistry to active caspase-3. No significant apoptosis was observed at 3h DLI, whereas the number of apoptotic epithelial cells increased from 6h to 24h DLI. Co-labelling of caspase-3 with the vessel marker collagen IV displayed apoptosis in endothelial cells only at 6h DLI. These results demonstrate that DLI mediates vascular or tumour apoptotic response. In conclusion, applying protocols that elicit Foscan® accumulation in cellular targets prone to apoptosis could be an efficient strategy to improve clinical outcome.

OC142

Hypoxia inducible factor as a molecular marker of response to photodynamic therapy with silicon phthalocyanine Pc 4

Elma D. Baron, Diana Santo Domingo, Andrew Hsia, Nancy L. Oleinick, Valdir Colussi, Kevin D. Cooper

Case Western Reserve University, Cleveland, OH, United States

We have shown that hypoxia inducible-1-alpha (HIF-1α) can potentially be a molecular marker for clinical response after photodynamic therapy (PDT) using silicon phthalocyanine Pc 4. In dermatology, PDT is currently being applied as a treatment for several hyperproliferative and malignant diseases. Silicon phthalocyanine Pc 4 is a second generation photosensitizer that more efficiently absorbs tissue-penetrating red light than previously-used photosensitizers. The photodynamic mechanism utilizes tissue oxygen to generate ROS such as singlet oxygen, which acts on cellular substrates-a process that potentially creates hypoxia in the tissue and also leads to apoptosis. These events can be monitored using HIF-1α as a hypoxic marker and caspase-3 as an indicator of early apoptosis. The purpose of this study was to examine changes in these two markers in CTCL patients-post PDT. Punch biopsies of CTCL lesions (n=5) and control/untreated tissues (n=5) were obtained 24 hours after treatment, and sections from these samples were immunostained using antibodies against HIF-1α and caspase-3. The percentage area of positive staining in the epidermis was determined using Image-Pro interactive image analysis software on 15 measures per sample. We observed a significant increase in caspase-3 in the epidermis after Pc 4-PDT. This increase differed significantly between clinical responders (17.9%) and nonresponders (4.5%) in nonresponders, ($p<0.001$). Similarly, a significant difference was noted for HIF-1α expression after Pc 4-PDT, such that 25.8% of the epidermis in responders showed positive staining versus 2.3% in nonresponders($p<0.005$). In conclusion, we have confirmed that Pc 4-PDT treatment leads to significant increases in caspase-3, and we demonstrate for the first time that HIF1-α expression increases after Pc 4-PDT. This increase is significantly more pronounced in clinical responders versus nonresponders.

OC143

Inhibition of EGFR-tyrosine kinase activity by photodynamic therapy

Anette Weyergang, Pål Kristian Selbo, Kristian Berg

The Norwegian Radium Hospital, Oslo, Norway

The photochemical reactions generated during photodynamic therapy (PDT) can be used for drug delivery as in photochemical internalization (PCI); a method for cytosolic release of macromolecular drugs. Many macromolecular drugs are taken up by the cells through the endocytic pathway and are sequestered in lysosomes were they are degraded before they have exerted their therapeutic effect. PCI is based on photosensitizers that preferentially localize in the membranes of endocytic vesicles. Light exposure causes membrane rupture and subsequent release of the drugs that are trapped in the lumen of these endosomes and lysosomes. It has been shown that both the specificity and therapeutic efficacy of PCI can be increased by linking the drug of interest to an epidermal growth factor receptor (EGFR) targeting moiety. However, the treatment outcome may be highly dependent on whether the photochemical reaction is performed before or after administration of the drug. It has been claimed that damage to EGFR can be important for PDT mediated cell death and that combination therapy with EGFR targeting drugs can increase the therapeutic outcome of PDT. It was therefore of interest to investigate whether the photochemical reactions utilized in PCI targeted EGFR.

This study reports on how PDT with the photosensitizer meso-tetraphenylporphine with two sulfonate groups on adjacent phenyl rings (TPPS2a) as used in PCI affects EGFR in NuTu-19 rat ovarian cancer cells in vitro. TPPS2a-PDT rapidly decreased the ability of EGFR to become phosphorylated upon EGF stimulation, and also attenuated total EGFR when a larger fraction of the photosensitizer was present at the plasma membrane at the time of light exposure. The mechanisms causing the photochemical effects on EGFR were further explored with experiments on EGF binding, receptor ubiquitinylation, endocytosis and cathepsin activity using Western blots stained with antibodies recognizing different regions of EGFR.

The results indicate that TPPS2a-PDT generated ROS directly oxidize regions of EGFR and that the Y1068 phosphorylation site at the intracellular domain of the receptor is the most sensitive target for TPPS2a-PDT. The importance of these results in relation to combination therapy strategies with PDT and EGFR targeting drugs will be discussed.

OC144

Inter- and intra-cellular signaling processes involved in PDT effect on neurons and glial cells

Anatoly B. Uzdensky

South Federal University, Rostov-on-Don, Russian Federation

Photodynamic therapy (PDT), a strong inducer of oxidative stress, is used for treatment of cancer including brain tumours. To study PDT effect on neuronal and glial cells (GC) we used a simple model object, isolated crayfish stretch receptor consisting of only two mechanoreceptor neurons (MRN) enveloped by GC. Photosens ($AlPcS_n$) and 633 or 670 nm laser were used for PDT treatment. PDT gradually inhibited and then abolished neuronal activity. Percent of necrotic MRN and GC progressively increased during next 8 h. Apoptosis of GC became evident 6-8 h post-PDT unlike MRN, whose apoptosis was not observed. Exogenous neurotrophic factors NGF or GDNF but not BDNF or heregulin HRG1-β1 protected GC but not MRN from PDT-induced apoptosis. NGF also protected GC from necrosis. Application of inhibitors or activators of diverse intracellular signalling pathways showed the involvement of calmodulin, CaMII and adenylate cyclase in PDT-induced necrosis of MRN and GC. Phospholipase C, protein kinase C and MAP kinase p38 were also involved in GC necrosis. PDT-induced necrosis of MRN and GC was reduced by phosphodiesterase. Phospholipase C participated in PDT-induced apoptosis of GC, whereas adenylate cyclase, JNK, protein kinases A and C protected GC from photoinduced apoptosis. The scheme of signaling pathways involved in MRN and GC responses to PDT was developed. According to obtained data, PDT-induced signaling processes differ in different cell types. Therefore, pharmacologic modulation of some signaling pathways may differently modify photosensitivity of different cells such as neurons and gliocytes, or possibly cancer and normal cells. This provide the basis for selective injury of malignant and protection of normal cells.

Supported by RFBR grant 05-04-48440.

IL145

The Photodermatoses - Non-carcinogenic responses to ultraviolet irradiation in man

John L M Hawk

St John's Institute of Dermatology, London, UK

The typical human skin responses to ultraviolet radiation (UVR) exposure are the largely DNA absorption and damage-led sunburning, pigmentation, hyperplasia, ageing and cancer, being respectively a repair process, attempts at the prevention of further damage and the results of cumulative non-prevented and non-repaired DNA damage. However, a host of other possible responses may follow cutaneous UVR absorption by chromophores other than DNA, against which the organism also has potential protective mechanisms, but which are at times inadequate. In such instances, any of a variety of so-called photodermatoses may supervene, but because DNA is not the initiating chromophore, there is generally no potentially carcinogenic outcome. Nevertheless, such reactions may be severely disabling, leading not infrequently to intolerable

itching and cosmetic disability, as well as precluding outdoor activities, while even milder forms are almost always significantly disruptive. Fortunately most can now be satisfactorily treated, generally enabling return to a largely normal lifestyle for sufferers. In more detail, these non-carcinogenic disorders comprise the immunological photodermatoses (polymorphic light eruption, actinic prurigo, hydroa vacciniforme, chronic actinic dermatitis and solar urticaria), the light-exacerbated dermatoses (particularly light-exacerbated seborrhoeic eczema) and drug and chemical photosensitivity (reactions to exogenous oral or topical preparations, or to endogenous porphyrins, leading to the porphyrias). Treatment for these conditions consists largely of sunscreen prophylaxis, immunosuppressive topical, oral or phototherapy, oral antihistamine therapy, drug or chemical avoidance, or the careful treatment of any underlying light-exacerbated dermatosis.

OC146

UV-exposure and skin-cancer: global variations in sensitivity and scenarios for the 21st century

Arjan van Dijk, Harry Slaper, Harm van Wijnen

RIVM, , National Institute for Public Health and the Environment, Bilthoven, Netherlands

We show three different scenarios for anthropogenic emissions of ozone depleting substances (ODS) and the associated risks for skin-cancer in the 21-st century. Our analysis of the source/risk chain is based on the model by Slaper et al. (Nature 384(6606), 256-258, 1996). Ozone scenarios are constructed from ODS emission scenarios via local trends in satellite-measurements for ozone and parameterizations for climate change dynamics. The resulting UV scenario is used in dose/effect relations for skin cancer based on the work by Scotto et al. (NCI NIH Pub. no. 83-2433, 1983), who studied white and moderately tanned people. We extended the single-point model by Slaper et al. to global scale maps. The time-span for trend-analysis has been increased by the coupled use of measurements from different satellite platforms. The ground-albedo algorithm has been improved and the fixed albedo in the original single-point model has been upscaled to 12 climatologic albedo maps. The original dose/effect relation, with only one unique sensitivity factor for all people, has been extended to global application with a global sensitivity model. This sensitivity model is based on skin-colour as follows: we combined the original parameterized dose/effect relations with observed cancer incidence data from a few hundred cancer registry bureaus from five continents. The resulting collection of skin-cancer related UV-sensitivity factors for these locations was related to local skin reflectivity. To reduce the influence of variations in quality of the data from different cancer registries, we estimated an underregistration factor for each bureau. This was done by exploiting the different non-linearities in the dose/effect relations for different types of skin-cancer. Global UV-related health-risks for the 21st century are presented.

IL147

Xeroderma pigmentosum, when the guardian of the gene pool goes on strike

Kenneth H Kraemer, Jennifer Boyle, Takahiro Ueda, Kyu Seon Oh, Kyoko Imoto, Deborah Tamura, Sikandar G. Khan, John J. Di Giovanna

Basic Research Laboratory, National Cancer Institute, Bethesda, MD, USA

Xeroderma pigmentosum (XP), and trichothiodystrophy (TTD) are rare, autosomal recessive human disorders with defective DNA repair. XP patients have a 1000-fold increased risk of developing sunlight induced skin cancers (basal cell carcinoma, squamous cell carcinoma and melanoma). TTD patients have sulfur deficient, brittle hair, skin sun sensitivity, dry scaling skin but no increase in skin cancer. XP has defects in 7 genes involved in nucleotide excision repair (NER) (XPA-XPG) and in an error-prone DNA bypass polymerase (pol eta). Surprisingly, TTD cells have (different) defects in some of the same genes (XPB and XPD). These genes are also components of basal transcription factor, TFIIH. NER proteins bind sequentially to sites of UV induced DNA damage. We used a UV microirradiation assay with cultured fibroblasts from XP and TTD patients. We found that mutations in NER genes will prevent translocation to the site of damage of the downstream components of the pathway. We have found that cells from patients with XP show delayed NER protein recruitment and persistence at UV damage sites. In contrast cells from TTD patients with different mutations in the *XPD* gene had reduced recruitment of NER proteins. These differences between XP and TTD in NER protein recruitment and persistence at sites of DNA damage may contribute to the increased UV induced skin cancer risk in XP patients.

IL148

Polymorphisms in DNA repair genes and cancer risk in the normal population

Steffen Emmert, Petra Laspe, Lars Boeckmann, Christiane Kuschal, Kai-Martin Thoms, Sandra Blankenburg, Karolin Zachmann, Ullrich Krueger, Holger Haenssle

Department of Dermatology, Georg-August-University Goettingen, Goettingen, Germany

Nucleotide excision repair (NER) removes a wide variety of bulky DNA lesions including UV-induced DNA damage. Defective NER results in three rare diseases: xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD). Because XP patients are skin cancer prone whereas CS and TTD patients are not, XP can be viewed as a model disease for skin cancer development including the development of cutaneous melanoma as well as basal and squamous cell carcinoma. These are the most common cancers in humans. Molecular-genetic diagnostics of patients with NER defects leads to a better understanding of the underlying genetics, transcriptomics, and proteomics of a clinical phenotype. Such phenotype-genotype studies also imply considerable consequences for the normal “healthy” population. NER gene polymorphisms or splice variants can lead to subtle variations in NER capacity and may serve as molecular markers for an increased skin cancer risk. For example, we found that some XPC gene polymorphisms are associated with a ~2-fold increased melanoma risk in the normal population whereas other XPC or XPG gene polymorphisms are not. Patients with an increased melanoma risk need to be informed about sunscreen measures and should be offered access to surveillance programmes. We, therefore, established an algorithm for the long-term follow-up of atypical nevi including digital epiluminescence microscopy which could then be offered to such high risk melanoma patients.

IL149

Large scale deletions of the mitochondrial DNA are associated with increased oxidative stress and altered cellular function in skin fibroblasts

Marc Majora, Tanja Maresch, Peter Schröder, Jean Krutmann

Institut für umweltmedizinische Forschung, Düsseldorf, Germany

Increased levels of deletions of the mitochondrial (mt) DNA are found in aged human tissues including the skin. It is currently not known how the presence of mtDNA mutations transduces into functional and structural changes of aged skin. Therefore we studied how human skin cells harbouring mtDNA mutations affect their microenvironment by seeding fibroblasts derived from patients suffering from Kearns-Sayre syndrome (KSS) and age/passage matched normal human fibroblasts (NHF) from healthy donors in 3D collagen gels resembling human skin.

Compared to NHF KSS fibroblasts had a 10.000fold higher level of mtDNA deletions examplified by quantitative measurement of the 4977bp Common Deletion. The increased level of mtDNA deletions in KSS fibroblasts was accompanied by a significant higher production of reactive oxygen species (ROS) in mitochondria and cytosol. Interestingly, KSS fibroblasts were capable of contracting a type I collagen gel faster and stronger than NHF. This effect was shown to be ROS dependent as the contraction difference between KSS fibroblasts and NHF could be reduced (i) in an anaerobic environment and (ii) in the presence of the antioxidant α-Phenyl-N-tert-butyl nitrone (PBN). Additionally, semi-quantitative real time PCR revealed a significantly higher expression of the lysyl oxidase (LOX), an enzyme involved in the cross-linking of collagen fibres, in KSS fibroblasts. Inhibition of LOX by β-aminopropionitrile reduced the contraction difference between KSS fibroblasts and NHF to a similar extent as PBN. As LOX has been shown to be a target gene of HIF-1α, we determined the expression of this hypoxia-related transcription factor. We found a twofold upregulation of HIF-1α at the mRNA level in KSS fibroblasts compared to NHF which was accompanied by upregulation of additional HIF-1α target genes like VEGFα, IL-8 which are also inducible by UV radiation. Stabilization of HIF-1α by $CoCl_2$ resulted in an accelerated contraction capacity of KSS fibroblasts while increased HIF-1α breakdown by the flavonoid apigenin resulted in a reduced contraction potential in both KSS fibroblasts and NHF.
We conclude that increased levels of mtDNA deletions are associated with increased oxidative stress which in turn may alter gene expression and cellular function in skin fibroblasts.

OC150

hOGG1 protein and gene are expressed more abundantly in the superficial than basal layer of human skin epidermis

Arash Javeri[1], Xiao Xuan Huang[1], Françoise Bernerd[2], Rebecca S. Mason[3], Gary M. Halliday[1]
[1]Melanoma and Skin Cancer Research Institute, Dermatology Research Laboratories, The University of Sydney, Sydney, Australia; [2]Life Sciences Research, L'Oreal, Centre Charles Zviak, Clichy, France; [3]Department of Physiology, Bosch and Melanoma and Skin Cancer Research Institutes, The University of Sydney, Sydney, Australia

Human 8-oxoguanine-DNA glycosylase (hOGG1) repairs 8-oxo-7,8-dihydroguanine (8-oxoG) which results from oxidation of guanine. Reactive oxygen species (ROS) formed in response to ultraviolet (UV) A radiation cause this DNA damage, which is involved in pathological processes such as carcinogenesis and aging. It is generally believed that the initiation of skin tumors requires penetration of solar UV to the actively dividing basal layer of the epidermis in order for acute damage to become fixed as mutations in the genome. In previous studies the majority of UVB fingerprint mutations have been found in the upper layers of human skin tumors, while UVA mutations have been found mostly in the lower layer. Our aim was to determine whether this localization of UVA-induced DNA damage is related to stratification of the repair-enzyme hOGG1. Anti-hOGG1 immunohistochemical staining of frozen sections of human foreskin, adult buttock skin, and reconstructed human skin samples showed the highest expression hOGG1 in the superficial epidermal layer (stratum corneum). Study of the hOGG1 mRNA expression again showed the highest level in the upper region of the epidermis. Our results indicate that weak expression of hOGG1 enzyme in the basal cells of the epidermis may lead to a lack of DNA repair in these cells and therefore accumulation of UVA oxidative DNA mutations.

IL151

Molecular solar energy conversion: lessons from photosynthesis

James Durrant
Imperial College London, London, UK

The photosystems of photosynthetic organisms are undoubtedly the most sophisticated examples of photochemical energy conversion. We now have a remarkably in depth understanding of the primary function of these photosystems, including consideration of their energy and electron processes and structure / function relationships. Recent advances in studies of water oxidation mechanism, lead in particular by the structural determination of PS II reaction centre, are allowing us to extend this understanding to the multi-electron chemistry necessary for solar fuel generation. These natural photosystems have for many years inspired chemists to synthesise artificial photosynthetic systems, such as molecular donor / acceptor systems and light harvesting arrays capable of emulating at least key elements of natural photosynthetic function. In parallel with these advances in artificial photosynthesis, attention has increasingly turned to the possibility of achieving practical solar energy conversion by developing chemical systems emulating at least some elements of natural photosynthetic function. Molecular based solar cells offer the potential for efficient solar energy conversion using low cost materials and fabrication techniques. Significant progress is now being made towards the commercial production of such devices for specific market applications. Similarly chemical water photolysis based on both molecular and inorganic materials and dual photoelectrodes analogous to the photosynthetic Z-scheme are maing rapid progress.
In my talk I will review some the progress being made in chemical solar energy conversion systems, focusing on the parallels with natural photosynthetic mechanisms, focusing in particular upon the work in my own group in dye sensitised photoelectrochemical solar cells and the photolysis of water to molecular oxygen and hydrogen.

IL152

Patterning light harvesting complexes onto self-assembled monolayers using photolithography

Christopher Neil Hunter
University of Sheffield, Sheffield, United Kingdom

Membrane proteins such as energy and electron transfer complexes, shuttles and pumps possess many desirable traits for the emerging field of nanobiotechnology, since they have evolved the capacity to absorb, transmit and interconvert various forms of free energy as electromagnetic radiation, redox potentials, and proton and ion gradients. Photosynthetic membrane proteins are particularly interesting since they operate on timescales ranging from femtoseconds to milliseconds, and with extraordinarily high efficiencies. The precision placement of the desired protein components on a suitable substrate is an essential prelude to any hybrid ‘biochip’ device, but a second and equally important condition must also be met: the retention of full biological activity. This work demonstrates the selective binding of an optically active membrane protein, the light harvesting LH2 complex from *Rhodobacter sphaeroides,* to patterned self-assembled monolayers at the micron scale and the fabrication of nanometre-scale patterns of these molecules using near-field photolithographic methods. Near-field photolithography has yielded rows of light-harvesting complexes only 98 nm wide. Retention of the native optical properties of patterned LH2 molecules was demonstrated using in situ fluorescence emission spectroscopy.

OC153

Photosynthetic complexes revealed by single particle analysis

Jon Nield
Imperial College London, London, UK

Transmission electron microscopy (TEM) and single particle analysis has revealed photosynthetically-active, membrane-bound protein structures from a variety of organisms in both two[1-4] and three[5,6] dimensions. The structures observed typically consist of either photosystem I or II with various peripheral light-harvesting components still bound. Such assemblies are often referred to in this context as 'supercomplexes' of one kind or another. In the first instance, preparations were isolated from a wide variety of organisms and conditions, in collaboration, and this included probing different strains and mutants in order to corroborate function with structural differences, be that water-splitting, solar capture or environmental adaptation. Supercomplexes were often seen to be highly labile entities in structural terms. This was not unexpected with >20 subunits present, being >700 kDa, having relatively large dimensions (320 Å to 385 Å in maximum length), and, especially in the case of photosystem II, a high turnover. Thus many tens of single particle datasets had to be built, extracted from TEM micrographs, and processed over the course of these projects. Each dataset (>10^4 protein complexes in 2D projection) was derived either from negatively stained samples or, in certain cases, non-stained vitrified samples[5,6]. The datasets were routinely 'computer purified', which involved CPU-intensive classification of the projections into characteristic view subpopulations derived from the many discrete 3D complexes present in any single preparation[7]. Negatively stained data resulted in 20 to 30 Å resolution maps, with cryo-TEM giving rise to 17 to 20 Å 3D density maps[5,6]. The latter data allowed for 3D modelling studies to open up new discussions regarding excitonic transfer routes or the localisation of oxygen-evolving centre subunits.

[1]Chen M., Bibby T., Nield J., Larkum A., Barber J. (2005) *FEBS Letts.* 579, 1306-1310. [2]Bibby T., Mary I., Nield J., Partensky F., Barber J. (2003) *Nature* 424, 1051-1054. [3]Bibby T., Nield J., Chen M., Larkum A., Barber J. (2003) *Proc. Natl. Acad. Sci. USA* 100, 9050-9054. [4]Kargul J., Turkina M., Nield J., Benson S., Vener A., Barber J. (2005)*FEBS J.* 272, 4797-4806. [5]Nield J., Morris E, Bibby T., Barber J. (2003) *Biochemistry* 42, 3180-3188 [6]Nield J., Barber J. (2006) *BBA* 277, 15006-15012. [7]Ruprecht J., Nield J. (2001) *Prog. Biophys. Mol. Biol.* 75, 121-164.

IL154

Variations in the organisation of chlorophyll protein complexes in oxyphotobacteria to exploit different ecological niches

Thomas S Bibby
National Oceanographic Center, School of Ocean and Earth Sciences, Southampton, UK

Oxyphotobacteria comprise all prokaryotic organisms that perform oxygenic photosynthesis and, as primary producers, represent an ecologically significant group of organisms. All oxyphotobacteria rely on two, highly conserved, light-induced reaction centres, the trimeric Photosystem I (PS I) and the dimeric Photosystem II (PS II), to perform photochemical reactions within the thylakoid membrane. To improve photosynthetic efficiency the photosystems are often associated with peripheral light harvesting antennae (peripheral antenna) that consist of a diverse array of protein and pigment complexes that allow specific species to thrive in different light and nutrient environments. To a first order it is this variety that describes different groups of photosynthetic organisms. We summarise how variations in the organisation of these complexes have enabled oxyphotobacteria to exploit different ecological niches and discuss the evolutionary relationships of the IsiA/Pcb family of pigment-binding proteins.

OC155

Carotenoid molecules in Photosystem II

Bruno Robert
Institute of Biology and Technology of Saclay (iBiTec-S) CEA Saclay, Gif-sur-Yvette, France

Photosystem II reaction centres bind two, non-equivalent, β-carotene molecules. Their electronic and vibrational properties exhibit significant differences, which are, up to now, unexplained. Both carotenoid molecules are redox-active, and can be oxidised by illumination of the reaction centres in the presence of an electron acceptor. The radical cation species show similar differences in their spectroscopic properties, suggesting that the oxidisation of these carotenoid molecules occurs in the absence of structural reorganisation of their immediate environment. These results will be discussed in the light of the properties of carotenoid molecules, either in organic solvents or bound to other photosynthetic proteins.

IL156

Photosystem II in the unusual chlorophyll d dominated cyanobacterium *Acaryochloris marina*

Alison Telfer
Imperial College London, London, UK

We have been studying an unusual natural system in order to investigate the energetic limitations involved in light-induced water splitting during oxygenic photosynthesis. *Acaryochloris marina* is a cyanobacterium whose pigment composition is dominated by chlorophyll *d* but also contains a small amount of chlorophyll *a* (<5% total). Chlorophyll *d* has an excited energy gap which is 0.09V less than the 1.8 V of chlorophyll *a* and it has been questioned whether chlorophyll *d* is energetically capable of carrying out charge separation in photosystem II. We have recently shown that primary charge separation in PS II probably occurs from chlorophyll *d* but that the positive charge is then stabilized on chlorophyll *a* (Schlodder, Cetin, Eckert, Schmitt, Barber and Telfer, *Biochim. Biophys. Acta*, 2007). This was demonstrated using time resolved absorption changes to measure the $P^+Q_A^-/PQ_A$ absorption difference spectrum in cells and a variety of different membrane preparations enriched, to different degrees, in PS II. As P740, the primary donor in PS I, was pre-oxidised with ferricyanide we could be sure that the 1-2 ms transient absorption decay was only due to $P^+Q_A^-$ recombination in PS II. Our results clearly show the involvement of both chlorophyll *a* and *d* in PS II electron transfer and supports observations by others that the redox potentials at the donor side of PS II of *Acaryochloris marina* are similar to those in chlorophyll *a* containing oxygenic organisms. Fluorescence decay measurements, indicative of charge recombination in PS II, show that there are modifications at the acceptor side consistent with the potential of the excited state being less reducing in *A. marina* as compared with chlorophyll *a* organisms (Vass, Cser, Telfer and Deak, 14th Photosynthesis Congress, Glasgow, 2007). The necessity for charge stabilization on chlorophyll *a* in PS II of *A. marina* despite the fact that a low energy chlorophyll *d* molecule carries out the primary charge separation step will be discussed.

IL157

The structural origin, biological function and biosensing applications of pH-sensitivity in beetle luciferases

Vadim R. Viviani[1], *F. G. C. Arnoldi*[2], *A. J. Silva Neto*[2], *Y. Ohmiya*[3]
[1]*Laboratorio de Biotecnologia e Bioluminescencia (UNISO), Universidade Federal de Sao Carlos, Sorocaba, SP, Brazil;* [2]*Depto Biologia, UNESP, Rio Claro, SP, Brazil;* [3]*Cell Dynamics Res. Group, National Institute of Advanced Science and Technology (AIST), Osaka, Japan*

Beetle luciferases produce a wide range of colors varying from green to the red. Among them, firefly luciferases display the

noteworthy spectral pH-sensitivity, which has been considered a denaturing side-effect. Despite many studies about the structure and function of firefly luciferases, the structural origin of pH-sensitivity remains unclear. Through a comparative site-directed mutagenesis study using a set of pH-sensitive (*Macrolampis*, *Cratomorphus*) and pH-insensitive (*Pyrearinus*, *Phrixotrix* spp) luciferases cloned in our laboratories, we started to investigate the origin of pH-sensitivity. Previously, we found that the conserved E354 modulates pH-sensitivity in firefly luciferases. Now we show that the loop between residues 223-235 is a major common structural determinant of bioluminescence colors. The residues Y/F/V227, G228 and N/T229 play key roles. This loop and the associated network of interacting residues constitute an alternative solvent gate to the active site in the pH-sensitive luciferases, modulating bioluminescence colors. Although pH-sensitivity has been considered a denaturing effect, recent evidences suggest that it may have a functional role in some fireflies. Finally, the spectral sensitivity of firefly luciferases to a variety of physical-chemical factors could be potentially useful to investigate dynamic intracellular events during *in vivo* assays. (Financial support: FAPESP and CNPq).

IL158

Structural insights into bioluminescent mechanism of calcium-regulated photoproteins

Eugene S. Vysotski[1,2], *John Lee*[2]

[1]*Photobiology Lab, Institute of Biophysics Russian Academy of Sciences, Siberian Branch, Krasnoyarsk, Russia;* [2]*Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA*

Ca^{2+}-regulated photoproteins are found in and are responsible for the light emission of a variety of bioluminescent marine organisms, mostly coelenterates. The best known of these are aequorin from jellyfish *Aequorea* and obelin from hydroids *Obelia.* The photoproteins are members of the large family of "EF-hand" calcium-binding proteins. The direct information concerning the mechanism of photoprotein bioluminescence derives from spatial structures of different ligand-dependent conformational states of obelin and aequorin determined by X-ray crystallography. The bioluminescence results from the Ca^{2+}-triggered decomposition of a high energy intermediate, the 2-peroxycoelenterazine molecule observed to reside in the binding cavity and stabilized in place by a network of hydrogen bonds. The movement of residues occurring as a result of accommodating Ca^{2+} in the nearby loops of EF-hands, would perturb these hydrogen bonds, destabilizing this intermediate and allowing the decarboxylation reaction that generates the product coelenteramide in its excited state. In the cavity structure of the Ca^{2+}-discharged photoprotein, a water molecule nearby the coelenteramide amide is so positioned that it could donate a proton to the intermediate dioxetanone anion, prior to its decomposition to the excited state product. Thus the primary product must be the neutral excited coelenteramide (light emission at 390-405 nm). The energetically feasible lower energy excited state (460-495 nm) observed from the natural photoproteins, results from the excited state proton transfer from the OH bound to the 6-phenyl substituent, to a nearby His residue as a proton acceptor. Based on research carried out over several years, this work has been supported by several sources: Office of Naval Research, National Institute of Health, Georgia Research Foundation and Georgia Research Alliance, Russian Foundation for Basic Research, "Molecular and Cellular Biology" program of the Russian Academy of Sciences.

IL159

Basis and application of ostracod bioluminescence

Yoshihiro Ohmiya[1], *Chun Wu*[2]

[1]*Hokkaido University, Sapporo, Hokkaido, Japan;* [2]*National Institute for AIST, Ikeda, Osaka, Japan*

In the post genome era, bioluminescent probes based on luciferase technology utilize both natural and quantitative light energy in comparison with fluorescent probes such as GFP has resulted in their ability to visualize the molecular diversity of genes and proteins in living cells. As our contribution to new luciferase technology, we have cloned a secreted-type luciferase (CLuc) from the marine ostracod Cypridina noctiluca, and demonstrated that the activity of CLuc in culture medium was remarkably higher than that of another ostracod luciferase. This high activity of CLuc is expected to be beneficial to the high-sensitivity and high-resolution monitoring of gene expression in living cells. In fact, we have developed real-time monitoring using secreted CLuc for a pharmacological assay that is based on targeted promoter activity. A model cell line was established with Rat-1 fibroblasts expressing CLuc driven by the promoter of a circadian clock gene, Bmal1. To accurately assay for temporally secreted CLuc activity, a perfusion culture was adopted in which the promoter activity was sequentially monitored by measuring the reporter activity in the perfusate. In trial studies, we demonstrated that the DEX-pulsed circadian oscillation was reasonably attenuated by RU486, a GC receptor antagonist, and that SP600125, a c-Jun N-terminal kinase inhibitor, caused phase shifting of the rhythmicity. The secreted ostracod luciferase presents a significant advantage as a non-destructible bioluminescent reporter. In addition, we developed a simple yet efficient method for the synthesis of (S)-ostracod luciferin. Furthermore, we created different (S)-ostracod luciferin analogues that emit light ranging from ultraviolet to green. The color difference in the ostracod bioluminescence system is expected to increase the potential for new applications.

IL160

Multicolor reporter gene technology for new multiplexed bioluminescent cell-based assays

Elisa Michelini[1], *Luca Cevenini*[1], *Laura Mezzanotte*[1], *Danielle Ablamsky*[2], *Tara Southworth*[2], *Bruce Branchini*[2], *Aldo Roda*[1]

[1]*Department of Pharmaceutical Sciences University of Bologna, Bologna, Italy;* [2]*Department of Chemistry, Connecticut College, New London, CT, USA*

The availability of new bioluminescent proteins, obtained by cDNA cloning and mutagenesis of wild-type genes, expanded the applicability of these reporters from the perspective of using more proteins emitting at different wavelength in the same cell-based assay.

In a proof of principle trial, we used two luciferases, a wild-type P.pyralis luciferase and a red thermostable mutant of L.italica luciferase, to develop a high throughput screening assay for bile-acid biosynthesis in a new cell-based perspective for high content assay. Bile-acid biosynthesis is in fact, a key determinant of intracellular cholesterol homeostasis and, in turn, cholesterol synthesis rate in hepatocytes. The two luciferases were expressed under the regulation of the promoter of one of the two main enzymes responsible for bile-acid biosynthesis, CYP7A1 or CYP27A1, and transfected into the hepatoblastoma cell line HepG2, stably transfected with an expression vector for Farnesoid X Receptor (FXR). In addition, a third reporter protein, Gaussia luciferase, was introduced as a vitality control. The activity of Gaussia luciferase was assayed by adding coelenterazine to the culture medium, which contained only the secreted Gaussia protein, thus avoiding interference with measurements of the two firefly luciferases. The firefly luciferases in cell lysates were assayed with beetle luciferin using a set of previously described optical filters.

We screened many potential inhibitors and determined that the maximum inhibition was achieved with chenodeoxycholic acid

(CDCA). The concentration of CDCA required to inhibit 50% of reporter activity (IC50) was determined to be approximately10 μM and 25 μM for CYP7A1 and CYP27A1, respectively.
The cell-based assay was performed in a high throughput 96-well microtiter plate format and required 20 hrs of incubation time of the cell suspension with the analyte in solution.
Good intra and inter-assay variability were obtained, 9 and 12 %, respectively. Hence, this "triple" assay, the first reported in literature, which employs either intracellular or secreted luciferases paves the way for the monitoring of multiple metabolic events for high-content screenings.

OC161

Bioluminescent enzyme-based biosensors

Valentina A. Kratasyuk[1], *Elena N. Esimbekova*[2]
[1]*Siberian Federal University, Krasnoyarsk, Russian Federation;* [2]*Institute of Biophysics, Siberian Branch of Russian Academy of Sciences, Krasnoyarsk, Russian Federation*

Principles of bioluminescent enzyme-based biosensors in which the effect of the sum of toxicants on bacterial bioluminescence parameters *in vivo* and *in vitro* is controlled are considered. Luminous bacteria and coupled and triple reactions catalyzed by FMN:NADH-oxidoreductase and luciferase are used in bioassays for environmental and medical monitoring. To estimate water quality, original bioluminescent biosensors have been devised and successfully used in ecological monitoring. The current situation in biosensors for monitoring of aquatic ecosystems is profoundly analyzed. This analysis is to provide a basis for the conception of creating a universal system of biosensors for ecological monitoring. The approaches to the creation of this system using bioluminescent organisms and their enzymes and to devise a laboratory model of a biosensors system are discussed. Since biosensors are used on vast territories, it is also essential to develop approaches to automation of the biosensors set. An automated set of biosensors including organisms and enzyme systems for the monitoring of the Yenisei River water is devised by selecting the bioassays that reflect the effect on the principal functions of living organisms. The work substantiates a new approach (use of series of model homologous compounds with the regularly changing physicochemical characteristics) to investigations of characteristics and fields of application of biosensors, conducted to estimate whether these bioassays should be included in the expert system. The system must contain the lowest possible number of biosensors sensitive to various groups of pollutants. Results of bioluminescent assays of waste waters from pulp and paper mills, natural sources and portable water in different regions of Altai and Krasnoyarsk Territories, salt water of the heath resort salt lake Shira (South Siberia) are considered. The possibility of using bioluminescent biosensors in medicine to control endotoxicosis degree during therapy has been developed. Bioluminescent assay may be used as a reliable criterion to monitor the course of disease for patients with bronchitis, ulcerous disease or chronic colecystitus. This method does not reveal differences in patients with sepsis, hepatocirrhosis or oncology. The methods are highly sensitive, rapid and simple and allow quantitative determination of the degree of seriousness of illness and estimation of the severity of a patient's condition.

OC162

Studies on the alteration in color of bioluminescence arising from *Vibrio fischeri* Y1

Hajime Karatani, Takashi Osaki, Masashi Yasui, Shoji Matsumoto, Shogo Ohta
Department of Biomolecular Engineering, Kyoto Institute of Technology, Kyoto, Japan

Marine luminous bacteria produce light in the luciferase reaction with $FMNH_2$, reduced form of riboflavin 5'-phosphate (FMN), molecular oxygen and an aliphatic aldehyde. The spectrum of bioluminescence (BL) is usually broad and the BL emission maximum occurs around 490 nm. However, some species alter the BL color due to the parcipation of accesary fluorescent protein in the luciferase reaction. *Vibrio fischeri* strain Y1 produces yellow light with a maximum at about 535 nm by virtue of endogenous yellow fluorescent protein (YFP). Interestingly, in logarithmic phase of the cell growth, *V. fischeri* Y1 cells change the BL color depending on the concentration of O_2 disolved in a cell culture. When the cell population is especially very high, *e.g.*, shortly before the culture enters the stationary phase, the BL color alteration is distinctly observed between in the presence and absence of air-supply to the culture. In relation to this BL color alteration, it was found that YFP fluorescence emission depends on the redox state of YFP-bound FMN. From the *in vitro* characterization using flavin reductase coupled luciferase reaction with dithionite reducing system, it was made clear that the reduced form of YFP no longer acts as a secondary emitter in the luciferase reaction, thereby resulting in the depression of yellow BL emission, and that the reoxidation of the reduced YFP reversibly regenerate the active form of YFP, leading to the retrieval of the original yellow BL emission. *V. fischeri* Y1 cells also exhibit an irreversible BL color alteration from blue to yellow during the cell growth, when the culture temperature is lower than 20 °C. The irreversible BL color alteration can be attributed to the change in the ratio of intracellular amount of YFP to that of luciferase. Besides YFP, *V. fischeri* Y1 carries a blue fluorescent protein (Y1-BFP), of which functional role in luciferase reaction is not made clear. However, Y1-BFP causes a blue shift in the *V. harveyi* luciferase reaction *in vitro*, where YFP is not active to trigger BL color alteration. Such a luciferase specufic sensitization with either YFP or Y1-BFP seems to be attributed to the difference in the protein-protein interaction. To evaluate the homology of YFP and Y1-BFP at the level of the primary structure of protein, the complete nucleotide sequence of the gene encoding Y1-BFP was determined and found to be 600 bp. The homology of YFP with Y1-BFP is approximately 42%. Both seem to have the sequence, corresponding to a ribityllumazine binding site, possibly suggesting that some functional relationship between YFP and Y1-BFP is present.

OC163

Energy conversion in bioluminescent reactions

Nadya S. Kudryasheva
Institute of Biophysics SB RAS, Siberian Federal University, Krasnoyarsk, Russia

Purpose of the work was to reveal general similarities and peculiarities of energy conversion in different types of bioluminescent reactions – marine bacteria, coelenteramides, and fireflies. Among similarities of the bioluminescent reactions are oxidative type of the reactions (with molecular oxygen included) and spectral-luminescent characteristics of emitting molecules: emitters of the bioluminescent reactions are characterized by effective fluorescence of $\pi\pi^*$- type and upper electron-excited states of $n\pi^*$- type. This suggests activity of the upper states, which can be formed as primary emitters in the oxidative bioluminescent reactions. The hypothesis on activity of the upper electron-excited states in bioluminescent process was experimentally verified using fluorescent molecules as energy acceptors. The hypothesis was confirmed in bacterial bioluminescence, however, activity of the upper electron-excited states was not found in firefly bioluminescence, probably because of highest efficiency of intramolecular energy transfer in the firefly emitter.
Quantum efficiency of bioluminescent reactions of marine bacteria, fireflies and marine coelenterates was found to decrease in the presence of heavy haloid atoms (Br or I) – so called 'external effect of heavy atom in bioluminescent reaction'. Two mechanisms were considered as responsible for the effect of heavy atom in the bioluminescent reactions: (1) physical effect on intramolecular energy transfer in the bioluminescent emitters and (2) biochemical

effect - inhibition of enzymatic activity. Conclusion was made on the main contribution of the biochemical mechanism to the effect of heavy atom in the bioluminescent reactions. Binding of heavy-haloid-compounds by the enzymes was confirmed in the polarized fluorescence experiments with a series of haloid-containing xanthenic dyes.
Peculiarity of bioluminescent spectra of marine coelenterates is based on their complexity: the bioluminescent spectra include spectral components of various forms of coelenteramide in a wide spectral region. The spectral components were determined, characterized and compared in bioluminescence and photoluminescence spectra of obelin, aequorin and cletyn. Process of proton transfer in the electron-excited states of the coelenteramide is responsible for contribution of the various components to the bioluminescence spectra, depending on aminoacid surrounding of the emitter in the enzymes.

PL201
Overview of clinical applications of PDT

Stephen G. Bown
University College London, London, United Kingdom

Awareness of the clinical potential of PDT is accelerating. From the world of overoptimistic enthusiasts, it is steadily expanding its rightful place in mainstream medical practice. Nevertheless, progress is slowed by regulatory hurdles, a relative lack of controlled clinical trials to prove efficacy and the reluctance of many doctors to adopt new treatments that require a radically different approach. Best established is the treatment of skin tumours (other than melanoma), where the cosmetic results are so much better than conventional treatment. PDT is now often the treatment of choice for all stages of mouth cancer, either as initial therapy or when other options fail. PDT is attractive for dysplasia in a range of hollow organs as it can ablate the mucosa without significant effect on the deeper layers, such as muscle, especially using ALA. Most important of these is Barrett's oesophagus (due to chronic acid reflux), for which PDT has been shown to significantly reduce progression to life threatening cancers. It is also effective for very early disease in the lungs, although at present few patients are diagnosed early enough for this to be applicable. New optical diagnostic techniques for pre- and post-PDT surveillance of high risk patients with dysplasia make the management of these patients easier. Interstitial PDT for tumours of solid organs involves light delivery by fibres inserted through needles positioned in the target organ under image guidance.This is gaining momentum, especially in the prostate, where focal therapy may be as good as complete gland ablation and with fewer complications. Unlike surgery or radiotherapy, PDT has the special attraction of being repeatable for recurrent disease. Using Tookad, light delivery starts before drug administration is complete, dramatically improving the convenience of PDT for staff and patients. For pancreatic cancers PDT also looks promising, certainly simpler and safer than major surgery. The efficacy of these treatments is markedly enhanced by developments in computer assisted image guidance and treatment monitoring. Outside oncology, there are early clinical trials to show that PDT can reduce the incidence of re-stenosis after balloon angioplasty to open up occluded arteries and that it can kill MRSA, the hospital "super bug", on infected skin ulcers. It has been established for the treatment of age related macular degeneration in the eyes for several years. There is also evidence of cost effectiveness: already there is firm data that the cost of PDT is less than half that of conventional therapy for head/neck cancers and for dysplasia in Barrett's oesophagus.

IL202
The relationship between erythemal and vitamin D solar exposure: spectra and doses

Robert M. Sayre[1,2]*, John C. Dowdy*[1]
[1]*Rapid Precision Testing Laboratory, Cordova, Tennessee, USA;* [2]*Division of Dermatology, Department of Medicine, University of Tennessee Center for the Health Sciences, Memphis, Tennessee, USA*

Recent and ongoing advancements in the study of vitamin D production by solar exposure has significantly increased our understanding of the complexity of the injury risk due to ultraviolet exposure versus the benefit of the vitamin D photosynthesized. Principal complicating factors include: how much effect does skin pigmentation have, how much skin needs to be exposed, how frequently are exposures needed and how best do we predict or determine how much vitamin D a single exposure or series of exposures produces? Conventional guidance and research has been mostly geared to preventing skin injury but not to determine how to safely and optimally obtain beneficial effects from ultraviolet exposure. One often hears that relative measurements of UV risk, such as the UV index, provide useful information. Many instruct that incidental outdoor exposure in the morning or evening can provide needed vitamin D. Some guidance even advises, erroneously, that adequate vitamin D may be synthesized while wearing high SPF 15-30 broad spectrum sunscreens. Unfortunately most of this guidance, while seeming to limit sunlight induced injury, will actually increase solar UV risk relative to desired vitamin D benefit. Furthermore, much of this guidance, if strictly followed, will reduce vitamin D production by solar UVB to inconsequential levels. Examination of the solar spectrum indicates that noon is the optimal time to expose as much of one's body as feasible to a limited suberythemal dose, perhaps ~1 SED, because this is when vitamin D effective UVB peaks. Clearly we are traversing a very fuzzy area of our understanding. It may yet be proven that the safest most effective way to make vitamin D in our skin is through controlled, spectrally appropriate, indoor exposures where practical full body exposure would greatly reduce requisite total exposure dose.

IL203
The relationship between exposure of ultraviolet radiation and vitamin D status

Ola Engelsen
NILU - Norwegian Institute for Air Research, Tromso, Norway

I review the main factors influencing the vitamin D status with a particular focus on ultraviolet radiation exposure and associated variables. Gaps of knowledge are discussed. Vitamin D is important for bone health, and may prevent some cancers e.g. in breast, prostate and colon. It may also inhibit some autoimmune diseases such as multiple sclerosis, diabetes-1, rheumatoid arthritis, etc. On the global level, the main source of vitamin D is the sun. Solar rays are in principle free, and toxic vitamin D levels from the sun are not possible. The effect of solar radiation on vitamin D synthesis depends to some extent on the initial vitamin D status. At moderate to higher latitudes diet becomes an increasingly important source of vitamin D because the sun is less intense and cold temperatures require more clothing. During winter, the UVB radiation is not strong enough to sustain any vitamin D synthesis at all. This period is referred to as the vitamin D winter. Despite this, the vitamin D status appears to improve with latitude in Europe, probably due to fair skin complexion and a generally higher vitamin D dietary intake. Only a few sources of food contain vitamin D, and those types of food are more part of the traditional diet in the north. For high latitudes and elevated desired levels of vitamin D intake the balance between skin burn and adequate vitamin D synthesis is not straightforward. Future projections of increased cloudiness, more migration of individuals with darker skin towards higher latitudes, as well as trends towards less outdoor

work and leisure activities form additional risk factors for vitamin D insufficiency.

Online calculators for computation of vitamin D synthesis in human skin are freely available on the internet (e.g. http://nadir.nilu.no/~olaeng/fastrt/VitD.html and http://nadir.nilu.no/~olaeng/fastrt/VitD_quartMED.html). I present some new sample results from those.

IL204

Solar UV-radiation, skin cancer and vitamin D: how much sunlight do we need?

Jörg Reichrath, Bernd Nürnberg, Wolfgang Tilgen

Dermatology Clinic, The Saarland University Hospital, Homburg, Germany

An increasing body of evidence shows a connection between vitamin D deficiency and independent diseases including various types of cancer. We tested the hypothesis whether patients that have to protect themselves against solar UV radiation for medical reasons, including patients with xeroderma pigmentosum (XP), basal cell nevus syndrome (BCNS), Lupus erythematodes (LE) or transplant recipients, are at risk to develop vitamin D deficiency. Basal serum 25-hydroxyvitamin D (25(OH)D) levels were analyzed in renal transplant patients (n=31), other transplant recipients (n=4), LE patients (n=3), XP patients (n=3), patients with BCNS (n=1), and in an age- and gender-matched control group at the end of winter (February/March). All transplant patients were advised after transplantation to avoid solar UV radiation by mechanical protection and by use of sunscreens. Basal 25(OH)D levels were compared using a non parametrical test (*Wilcoxon rank sum test*). P<0,05 was considered significant. Basal serum 25(OH)D levels were significantly lower in renal transplant recipients as compared to controls (p=0.007). Geometric mean (with 95% confidence interval) in renal transplant patients was 10.9 ng/ml (8.2-14.3) compared to 25.1 ng/ml (15.7-25.5) in controls. Other transplant recipients and patients with XP, BCNS or LE were vitamin D deficient as well. We demonstrate that these patients are at high risk to develop vitamin D deficiency, that has to be substituted (e.g. via oral treatment) to protect against serious vitamin D deficiency-related health problems without increasing the risk to develop solar UV-induced skin cancer. Additionally, our finding that protection against solar UV radiation causes vitamin D deficiency underlines the need for re-defining dermatological recommendations for solar UV protection in skin cancer prevention programs.

IL205

Solar UVR, vitamin D and prostate cancer: how strong is the relationship?

Richard C Strange

Keele University Medical School, Hartshill Campus, Guy Hilton Research Laboratories, Stoke-on-Trent, Staffordshire, United Kingdom

Common diseases appear to result from poorly understood interactions between genetic and environmental factors. While many factors and genes have been studied in this regard, relatively few such interactions have been unequivocally linked with disease risk or outcome. Recent studies have implicated sunlight in the pathogenesis of various diseases. Thus, humans are exposed to ultraviolet radiation (UVR) throughout their lives and have developed phenotypes that mediate the deleterious and beneficial effects of this exposure. The harmful effects include formation of products that can give rise to mutations in key genes. These findings form the molecular basis for the accepted view that inappropriate exposure increases risk of skin cancers such as basal cell carcinoma (BCC). However, while UVR is a key causative factor, establishing what constitutes inappropriate exposure is less certain. It is also recognised that UVR exerts beneficial effects including initiation in skin of the 1,25 dihydroxy vitamin D synthetic pathway. Adequate synthesis of 1,25 dihydroxy vitamin D is important since in concert with the vitamin D receptor, the vitamin exerts a key role in numerous pathways via mediation of the expression of around 200 genes. It is of public health interest therefore, that hypovitaminosis is common worldwide. These findings have contributed to the idea that low levels of exposure to sunlight contribute to an increased risk of various diseases including some cancers.

Prostate cancer has been intensively studied in this regard. Thus, ecologic studies reported a north-south trend in United States prostate cancer mortality rates during 1950-1994 that was inversely correlated with exposure. Further ecologic studies based on data from various countries found UVR was protective against mortality from the cancer. We have reported in a series of case-control studies that measures of exposure such as regular holidays abroad and sunbathing are inversely associated with prostate cancer risk and cases with the lowest quartile of exposure developed the cancer significantly earlier than other men. Independent studies have shown that high residential exposure is linked with reduced risk of fatal prostate cancer. A review of 3 ecologic, 3 case-control and 2 cohort studies concluded there was an inverse correlation between UVR and prostate cancer risk and mortality. We have also found associations with outcome; exposure during adult life is inversely linked with risk of tumours with advanced T-stage. Roles for skin type and genetic factors associated with this phenotype and vitamin D synthesis and handling in determining prostate cancer risk and outcome have also been identified. However, while a variety of studies indicate that chronically low exposure to sunlight predisposes to prostate cancer, other cancers and, non-cancerous diseases including multiple sclerosis by a vitamin D-mediated mechanism, the hypothesis is unproven. Indeed, other explanations are possible. However, given the public health implications of the hypothesis and potential for developing novel therapeutic modalities we believe the concept is worthy of further investigation.

OC206

Sun beds and cod liver oil as vitamin D sources

Johan Moan[1,2], Alina Carmen Porojnicu[1]

[1]Rikshospitalet Radiumhospitalet HF, Oslo, Norway; [2]Department of Physics, University of Oslo, Oslo, Norway

We wanted to evaluate sun beds as vitamin D sources for humans and to check if the recommended daily intake of 200 IU of vitamin D is sufficient to keep up a good vitamin D status. Volunteers were exposed to suberythemal doses from a commercial sun bed for five weeks during winter time. This led to a large increase in serum calcidiol in all volunteers, from typical winter values to typical summer values. After the five weeks half of the persons were given a daily supplement of 200 IU vitamin D in the form of cod liver oil pills, while the other half of the persons acted as controls with no supplements of vitamin D. The calcidiol level in both subgroups decayed at the same speed and reached the pre – sun bed values after about six to eight weeks. We conclude that moderate doses of UV radiation from commercial sun beds is a good source of vitamin D comparable with solar radiation, and that the recommended intake of vitamin D (200 IU/day) is too small to keep up a typical summer level of serum calcidiol during winter time.

OC207

Does casual exposure to UK sunlight provide sufficient vitamin D levels?

Marie T Durkin[1], Richard Kift[2], Lucy V Bunhill[1], Donald Allan[1], Jacqueline L Berry[3], Ann R Webb[2], Lesley E Rhodes[1]
[1]Photobiology Unit, Dermatological Sciences, University of Manchester, Manchester, United Kingdom; [2]Earth, Atmospheric & Environmental Sciences, University of Manchester, Manchester, United Kingdom; [3]Endocrine Sciences, University of Manchester, Manchester, United Kingdom

Solar ultraviolet radiation (UVR) is carcinogenic, but is the major source of vitamin D, which is essential for optimal bone health and linked with other aspects of health. Brief periods of exposure of limited skin surface areas to sunlight have been proposed to be sufficient for vitamin D acquisition; our objective is to examine this assumption.

60 healthy white Caucasian subjects (39 F: 21 M, mean age 37 yrs, range 20-59 yrs, skin type I-III) were given a course of UVR exposure designed to mimic casual exposure to sunlight during a British summer. Wearing T-shirt and shorts they were exposed to 1.3 SED of UVR 3 times weekly for 6 weeks, in a total body cabinet fitted with fluorescent lamps emitting a UVR spectrum similar to sunlight (Philips Cleo Natural and Arimed B lamps, emission 290-400 nm). The course was administered in January and February, i.e. at a time when vitamin D attributable to ambient UVR is negligible. Blood was sampled weekly and assayed for 25 hydroxyvitamin D (25OHD) by HPLC with UV absorption; data is currently available on 15 subjects.

Mean 25OHD levels increased from 14.6 (SD 6.52) ng/ml at baseline, to 25.7 (6.06) ng/ml at the end of the course, p<0.0001. Before commencing UVR treatment, 25OHD levels below the levels for deficiency (<5 ng/ml), insufficiency (<20 ng/ml), and the proposed optimal level (32 ng/ml) were found in 1 (7%), 11 (73%), and 3 (20%) individuals, respectively. After the UVR course, levels below those for deficiency, insufficiency and the proposed optimum were found in 0 (0%), 3 (20%) and 10 (67%) individuals respectively, while 2 (13%) individuals reached levels >32 ng/ml.

Hence, in this sample, the majority of white Caucasian volunteers had 25OHD levels in the insufficiency range during the winter months. While a course of UVR designed to mimic casual sunlight exposure through a British summer approximately doubled the 25OHD level, insufficient levels persisted in some volunteers, and few achieved the proposed optimum. This will be further explored in a larger subject population.

This research was funded by Cancer Research UK.

OC208

Dose dependent effect of sunbed radiation on cutaneous vitamin D synthesis in humans, a randomized controlled trial

Elisabeth Thieden[1], Henrik L. Jørgensen[2], Niklas Rye Jørgensen[3], Peter A Philipsen[1], Hans Chr. Wulf[1]
[1]Department of Dermatology, D92, Bispebjerg Hospital, University of Copenhagen, Copenhagen NV, Denmark; [2]Department of Clinical Biochemistry, Bispebjerg Hospital, University of Copenhagen, Copenhagen NV, Denmark; [3]Department of Clinical Biochemistry, Hvidovre Hospital, University of Copenhagen, Copenhagen NV, Denmark

Objective: To investigate in a controlled, randomized, open study if use of modern sunbeds with sunlamps emitting mainly UVA and only 0.5% or 1.4% UVB will increase the serum level of 25OH VITD, if so, is the 25OH VITD level dependent on the UVB dose?

Method: 41 Caucasian, healthy, females 50+ were randomized to receive sunbed treatment with sunlamps emitting 0.5% UVB (n=20) or no treatment (controls, n=21). Most of the controls accepted to continue with sun bed treatment (UVB 1.4% group, n=15). The subjects in the two treatments groups (UVB 0.5%) and (UVB 1.4%) received 8 sunbed sessions wearing panties. 4 sessions of 6. min.: day 0, 2, 4, 7 and 4 sessions of 12 min: day 9, 11, 14, 16. Serum concentrations of the following blood parameters were assessed to identify possible differences in serum levels of 25OH VIT D; (PTH); Ionized calcium; Alkaline phosphatase at (t=0), (t=9, before 5th sunbed session) and (t=18).

Results: The increase in serum 25OH VITD from (t=0) to (t=9) was in average: 25% in the UVB 0.5% group, from 49.4 nmol/l to 59.4 nmol/l or (12 nmol/l, (SD 11 nmol/l, range -9 - 39 nmol/l), p=0.0002) and 58% in the UVB 1.4% group, from 45.9 nmol/l to 72.5 nmol/l or (27 nmol/l (SD 9 nmol/l, range 11 – 46 nmol/l) p<0.0001). There was a further but not significant increase in serum 25OH VITD from (t=9) to (t=18) in average: 4% in the UVB 0.5% group to 62.0 nmol/l or (3 nmol/l (SD 9 nmol/l, range -16 - 30 nmol/l), p=0.2) and 0.8% in the UVB 1.4% group to 75.3nmol/l or (0.6 nmol/l (SD 18nmol/l, range -36 - 33 nmol/l), p=0.9). No significant differences in serum 25OH VITD were found from t=0 to t=18 in the control group. No significant decrease in PTH was found from (t=0) to (t=9) in any of the groups and from (t=0) to (t=18) in the UVB 1.4% group only from 4.0 pmol/l (SD1.8) to 2.6 pmol/l (SD 0.9). No significant differences in ionized calcium or alkaline phosphatase were found in any of the groups from (t=0) to (t=18). Increasing with UVB dose and exposure time 37% to 69% of the treated subjects had side effect as erythema or sun allergy.

Discussion: Even the little UVB (0.5% and 1.4%) emitted was enough to increase the 25OH VITD serum level but a plateau serum level was reached already after 4 sessions. The plateau serum level was UVB dose dependent. We do not recommend sunbed use to increase serum 25OH VITD due to the carcinogenicity of UVB and the high frequency of acute side effects.

IL209

ALA and its clinical impact, from bench to bedside

Barbara Krammer
University of Salzburg, Department of Molecular Biology, Salzburg, Austria

Fluorescence detection with ALA is used in a variety of (pre)malignant and non-malignant diseases for diagnosis (ALA-FD) and for fluorescence-guided resection. ALA is also applied in photodynamic therapy (ALA-PDT) of superficial (pre)malignant lesions in the fields of dermatology, urology, neurosurgery, otorhinolaryngology, gynecology and gastroenterology.

20 years ago the use of ALA for PDT and FD was established by Z. Malik, H. Lugaci, J. Moan, J. Kennedy and R. Pottier. Since a few years ALA is approved as Levulan for actinic keratoses (EU, US), and the ALA-ester Metvix for basal cell carcinoma (EU).

ALA is a precursor of the potent photosensitizer protoporphyrin IX (PpIX) in the heme biocycle. If ALA is given externally to cells, they generate PpIX in excess, which cannot be quickly converted to the end product heme and therefore remains available as sensitizer for a limited time. During this time, the accumulated PpIX has to be irradiated with red light of a wavelength of 635 nm provided by lasers, lamps or LED sources in the presence of oxygen.

For patient treatment, several factors have to be considered. Administered mainly in topical or oral form, ALA penetrates tissue only in a sub-optimal way, which is currently improved by debulking tumors, iontophoresis or repeated treatment, but also partially by the use of ALA-esters (Metvix). PpIX is not only accumulated higher in many malignant vs. normal cells, but also in several abnormalities and in mucosa. It may be found in macrophages, dendritic cells and activated lymphocytes.

If ALA is administered to the patient for at least 3 hrs, sufficient PpIX is accumulated selectively in target cells for irradiation, which may be accompanied by a burning sensation on the treated area. Due to a saturation process of PpIX formation and a rapid photobleaching during irradiation, the risk of overdosage by the clinician is relatively low. Pharmocokinetical studies demonstrated a low systemic photosensitivity and an excretion of PpIX via natural routes. However, immune reactions to ALA-PDT could be found.

OC210
Molecular signaling in cancer cells after PDT and its clinical relevance
Charles Gomer
University of Southern California, Los Angeles, California, USA
Therapeutic applications of photodynamic therapy (PDT) for cancer treatment remain encouraging but improvement of long-term PDT responsiveness is needed to decrease tumor recurrences. A growing number of clinically relevant molecular and cellular responses that can modify treatment efficacy are observed following PDT. Oxidative stress and tissue hypoxia are induced by PDT and these reactions can elicit the transcriptional and/or translational expression of genes associated with inflammation, angiogenesis, immuno-modulation, apoptosis, and signal transduction. Specifically, preclinical studies have shown that PDT can induce expression of pro-survival pathways involving vascular endothelial growth factor, cyclooxygenase-2, prostaglandins, tumor necrosis factor, matrix metalloproteinases, various interleukins, Akt, survivin, and stress proteins. This presentation will provide an overview of the current information on PDT mediated signal transduction and activation of a survival phenotype in PDT treated cancer cells and tissue. Methods to enhance PDT by blocking the upregulation of selective signaling molecules will also be presented.

IL211
Current overview of clinical photodynamic application in neurosurgery
Sadao Kaneko
Kashiwaba Neurosurgical Hospital, Sapporo, Hokkaido, Japan
The prognosis of the malignant brain tumor patients is extremely pessimistic, because median survival time of GBM is only about 16 months despite all available therapy. Photodynamic medicine including PDD, PDT and fluorescence guided tumor resection is very promising for the treatment of malignant brain tumors. We have applied to 230 brain tumor patients for photodynamic diagnosis and 60 malignant brain tumor patients for photodynamic therapy. Especially, We have applied 11 patients that photodynamic therapy following Photodynamic diagnosis were administered for the malignant glioma patients at or near the eloquent brain areas and spinal cord tumor patients to get a good prognosis and to avoid a dysfunction of the patients.
I would like to talk on clinical results of this study and the current overview of photodynamic medicine in neurosurgery.

OC212
Photodynamic therapy and fluorescent diagnostics in clinical settings of Russian Cancer Research Center
E.G. Vakulovskaya, B.K. Poddybny, Y.P. Kuvshinov, G.V. Ungiadze, V.B. Karakhan, V.V. Kuznecov, V.V. Ashmarov
Russian Cancer Research Center of Academy of Medical Sciences of Russia, Moscow, Russia
Introduction: We have clinical (more then 1000 patients) and experimental experience in fluorescent diagnostics (FD) and photodynamic therapy (PDT) in cancer patients with different second-generation photosensitisers (Photosense (PS), Alasense (AS), fotoditazin (FD), Radachlorin (RC)) at Russian Cancer Research Center from 1993 in frames of clinical trials.
Aim: working out methods of PDT with second-generation sensitizers, evaluating the efficacy of it in patients with different cancer localizations.
Materials: PDT and FD with PS, AS, FD, RC was done in more the 1000 patients with different localization of tumor (head and neck cancer (HNC), gastrointestinal cancer (GIC), breast cancer, gynecological cancer, bladder cancer, brain tumors). Detecting of tumor borders, intensity of accumulation of photosensitizers in tumor, normal tissues were done by Spectral-fluorescent Complex and fluorescent endoscopes. We used semiconductive lasers for PDT: Biospec- 672, Atkus-2, Milon -660. Regimes with multiple irradiation were worked out for PS with total light dose till 400-600 J/cm^2 and single irradiation with light dose 100 - 300 J/cm^2 using PD and RC, sensitizers were injected intravenously in doses 0.4-0.8 mg/kg and 0.7-2.4 mg/kg respectively. AS was applied topically, given per os or instilled.
Results: FD provided information about disease advance, allowed identification of subclinical lesions and PDT control, demonstrated high sensitivity (98,7 - 100%), and specificity (78,3 - 98,1%). After PDT with PS in HNC we've had complete response (CR) in 76.0%, partial response (PR) in 21,9% of patients, after PDT with RC and FD in skin cancer CR in 71,4% and 90%, PR – in 28,6% and 10% patients respectively. Cancer of larynx, cancer of low lip T1-3N0M0 were more sensitive to PDT - CR 90 % and 76,5%. Main side effect with PS is increased skin sensitivity to sunlight, with FD and RC it's short-term (4-5 days).
Conclusion: Our experience show pronounced efficacy of PDT in cancer patients both as radical (HNC, GIC) and palliative treatment (GIC, HNC, breast cancer). FD is providing significant information about borders of tumor growth, allowed identification of subclinical lesions, demonstrated significantly higher sensitivity and specificity comparing traditional white-light diagnostic procedures.

IL213
PDT - Registered and future indications in dermatology
Alexis Sidoroff
Medical University of Innsbruck, Department of Dermatology and Venereology, Innsbruck, Austria
Photodynamic Therapy (PDT) has been used for dermatological conditions more than 100 years ago. Since the introduction of topically applicable 5-aminolevulinic acid (5-ALA) in the 1990's this treatment modality experienced its revival in modern dermatology. Registered 5-ALA preparations (Levulan ®) and ALA methylester (Metvix ®) have lead to broader use of this therapeutic option. In many countries topical PDT can now be used in the treatment of actinic keratoses (AK), Bowen's disease, and basal cell carcinomas (BCC). PDT has proven to be efficacious, cure rates are comparable to other treatment modalities and in general PDT delivers excellent cosmetic results. In special clinical constellations (like in Bowen's disease in areas where surgery would be difficult and/or lead to unsatisfactory cosmetic results) PDT can nowadays be regarded as first line treatment. PDT thus should be part of the therapeutic spectrum wherever patients with non melanoma skin cancer (NMSC) are been taken care of.
In addition to that PDT has been used on an experimental basis in the treatment of a vast range of different cutaneous affections like diseases of the pilosebaceous unit (e.g. acne and rosacea), inflammatory skin diseases (e.g. psoriasis, pseudolymphoma, sarcoidosis, lichen planus, or Darier's disease), cutaneous sclerosis (e.g. localized scleroderma, lichen sclerosus), infectious skin diseases (viral warts, cutaneous mycoses, leishmaniosis, antibacterial PDT), and skin rejuvenation. Mechanisms of action and treatment parameters may vary and have yet to be optimized, but the beneficial potential of PDT beyond NMSC has been demonstrated. Although many results are promising, basic and clinical research (e.g. development of new sensitizers or adapting illumination parameters) is still needed to broaden the spectrum of dermatological indications for PDT in the future.

IL214
PDT for cholangiocarcinoma: review and personal experience
Steve Pereira
University College London, London, UK
Cholangiocarcinoma and carcinoma of the gall bladder are tumours of the biliary tract that are considered as one pathological entity (biliary tract carcinoma, BTC). Worldwide, BTC is the second most

common primary liver cancer after hepatocellular carcinoma, accounting for 15% of all primary hepatic malignancies. Recent epidemiological data from around the world have shown a steady and steep rise in mortality rates from intrahepatic cholangiocarcinoma over the past 20 years. The cause of this rise is unknown and does not appear to be explained simply by improvements in diagnosis or changes in coding practice.

BTC has a poor prognosis, with similar incidence and mortality rates and an overall five-year survival of less than 5%. Surgery is currently the only curative treatment but is limited to tumours that have not extended beyond the first division of either the left or right hepatics ducts (approximately 20% of cases), with 5-year survival rates varying from 9-30% in selected series. The remaining 80% of cases require palliation of symptoms as part of a multidisciplinary approach. Although most patients can be palliated promptly by endoscopic or percutaneous placement of one or more biliary stents, the prognosis remains poor, with complex hilar lesions having a median survival of less than six months. Since the cause of death in BTC is commonly due to recurrent biliary obstruction and intrabiliary sepsis rather than metastatic disease, a key issue of palliative therapy is that of control of locally progressive disease and optimal biliary drainage.

PDT may represent a paradigm shift in the palliative care of patients with BTC. Clinical data to date indicate that PDT for cholangiocarcinoma is well tolerated with low toxicity. In case series and two small randomized studies, PDT plus biliary stenting improves serum bilirubin levels, quality of life and patient survival compared with historical or matched controls. A multicentre phase III study (PHOTOSTENT-02) is currently underway which should further define the role of PDT in patients with locally advanced or metastatic BTC. The potential role of PDT as a neoadjuvant to increase R0 resection rates, and multimodal treatment approaches incorporating PDT, chemotherapy and novel biological therapies, will also be discussed.

OC216

Cancer therapy using Vascular-Targeted Photodynamic therapy (VTP) with TOOKAD

Yoram Salomon, Avigdor Scherz

The Weizmann Institute of Science, Rehovot, Israel

In Vascular-Targeted Photodynamic Therapy (VTP), the photosensitizer drug is illuminated while circulating in the tumor vasculature. Consequently, cytotoxic reactive oxygen species (ROS; mainly super oxide and hydroxy radicals) are generated, inducing intravascular damage that culminates with complete blood stasis within minutes. Downstream tumor tissue becomes ischemic with consequent development of necrosis and tumor eradication within 2-3 weeks, as observed in preclinical tumor models. Using fluorescent intravital microscopy and MRI in xenograft models in mice and rats, we have explored the hemodynamic response to VTP. It was found that immediately upon illumination, the blood flow in the tumor arterioles slows down along with the induction of vascular constriction and coagulation. Venous blood flow presents a similar phenomenon, but with a slight delay. Using a 10min VTP treatment protocol, blood stasis in the tumor was found to remain reversible in the first 5 minutes (if the illumination was turned off) but became committed to irreversible stasis towards the end of the protocol. It was further shown that tumor blood vessels are appreciably more sensitive to VTP compared to the vasculature of normal tissue. This treatment approach is presently being applied in clinical trials for treatment of prostate cancer, using TOOKAD, a drug invented in our laboratory that is currently being tested by Steba Biotech in clinical trials in several countries. TOOKAD is the first of a family of Pd-bacterochlorophyll-based sensitizers selected for VTP of patients with recurrent or primary localized prostate cancer. Information that points at efficacy and selectivity of TOOKAD-VTP will be presented along with highlighting data from the clinical trials.

Supported by STEBA-BIOTECH and NEGMA, France.

IL215

25 years of PDT in neurosurgery: a review

Herwig Kostron

Department for Neurosurgery, Medical University Innsbruck, Innsbruck, Austria

Malignant brain tumors carry almost always a lethal prognosis for the patients despite all available therapies. Photodynamic therapy (PDT) are intensively investigated as adjunctive therapy modality for malignant brain tumors due to their high therapeutic ratio. Photodynamic Diagnosis (PDD) and fluorescence guided tumor resection was also recently added to this armentarium.

Since 1982 PDT had been adopted for neurosurgery in various laboratories by the author. Various photosensitizers as well as incoherant and coherent light sources were used to induce the PDT effect. Direct intratumoral injection of HP, light and ionizing radiation proved to be curative in a rat brain tumor model.

In 1985 the first patient was treated with a glioblastoma multiforme with 15 J , this patient responded nicely and he lived for 6 months without any other treatment.. In the following years over 100 patients with malignant brain tumors were treated after sensitisation with various formulations of HP, such as HPD, Photosan, and Photofrin. The results were encouraging , patients living significantly longer with 19 to 24 months for primary and up to 15 months for recurrent glioblastomas. The sensitisation with the second generation PS mTHPC (Foscan) did however not prove to be of advantage.

The introduction of mTHPC-induced fluorerscence guided resection and intraoperative diagnosis gave significant advantage for tumor resection, which translates directly to improvement of survival.

In conclusion, PDT and PDD had proven to be a valuable addition to the armentarium in brain tumor therapy with a most favorable side effect profile.

OC217

Cellular and molecular effects of a new chlorine based photosensitizer in human cancer cells after light exposure

Heinrich Walt

Research Division of Gynecology, Department of OB/GYN, University Hospital, Zurich, Switzerland

Photodynamic therapy (PDT) of cancer is based on the preferential uptake of a photosensitizer (PS) into cancer cells, and its activation at a specific wavelength. The reactive oxygen species produced by this activation promote apoptosis and necrosis in the affected cells. The uptake, distribution and effects of a new PS would therefore be important for target identification, optimal time of illumination and molecular analysis.

Accumulation of the liposomal PS Foslipos® (Biolitec AG, Jena, Germany) was observed for 15 min, 1h, 2h, 3h, 4h and 5h with confocal laser scanning microscopy in a human prostate cancer cell line (PC-3). Following a 5h exposure to Foslipos, PDT was performed (652 nm, 1 Joule/cm^2). After 15min, 1h, 2h, 5h and 24h, RNA was extracted and RNA quantity and quality was determined. A similar experimental set was applied in parallel with a colony forming assay (CFA).

First fluorescence signals of Foslipos appeared after 15 min exposure, while the maximum of PS detection was observed after 5h. In both the PDT-treated and the control samples, RNA amounts remained constant over time. However, RNA quality was clearly affected in experimental samples: in contrast to controls, a continuous and statistically significant RNA degradation (ratio of 28S:18S) was measured between early and late time-points in cells after PDT. CFA results showed continuously less colonies between 5h and 24h.

Knowing the exact time points of PS accumulation is essential for PDT. Furthermore do our results demonstrate that desintegration of RNAs starts already within the first five hours after light exposure.

These early parameters of RNA desintegration will help us to better understand molecular events during PDT.

OC218

Monitoring ALA and mTHPC-PDT during intraluminal and interstitial PDT

Bastiaan Kruijt, Angelique van der Ploeg-van den Heuvel, Slavka Kascakova, Arjen Amelink, Henricus J.C.M. Sterenborg, Dominic J Robinson

Center for Optical Diagnostics and Therapy, Department of Radiation Oncology, Erasmus University Medical Center, Rotterdam, The Netherlands

The therapeutic effect following PDT depends on a combination of parameters that include drug dose, drug-light interval, and light fluence (rate). These parameters are in general only investigated in pre-clinical models and some stage I/II clinical trials optimizing PDT. During clinical ALA and Foscan-PDT we observe large variations in fluence rate during therapy that influences the delivered light fluence. The underlying cause of these variations in fluence rate and their influence on the delivered PDT dose are unknown. Variations in in-situ fluence rate, photosensitiser photobleaching and tissue optical properties were monitored during ALA-PDT in the rat oesophagus and during Foscan-PDT in muscle overlying the rat abdomen. Local variations in tissue oxygenation, blood volume and scattering intensity were monitored using Differential Pathlength Spectroscopy. We investigated the inter-relationship of these parameters for a wide range of light fluence rates. These data were compared to the response following PDT in the oesophagus and to data acquired during clinical Foscan-PDT for head and neck cancer. Changes in light fluence rate during PDT were strongly influenced by changes in tissue optical properties caused by changes in light scattering intensity, oxygenation and blood volume. These effects were strongly fluence rate dependent and not always predictable. We found that monitoring fluorescence photobleaching of PpIX and Foscan while simultaneously monitoring the local saturation during therapy could be used as PDT dose metrics but that this was very challenging for interstitial PDT. We present an overview of how these dosimetric data might be incorporated into treatment planning protocols for intraluminal ALA-PDT.

OC219

Determination of an action spectrum for the suppression of cutaneous recall immune responses in humans

Yasmin J Renwick, Tai A Phan, Gary M Halliday, Diona L Damian

Department of Dermatology, The University of Sydney, Sydney, NSW, Australia

Introduction: Ultraviolet radiation (UVR)-induced suppression of cutaneous immune responses enhances the development of skin cancers. We determined the immune suppressive effectiveness of various narrow bandwidths across the UVR spectrum in order to calculate the relative immunosuppressive contribution of UVB (290-320nm) and UVA (320-400nm) in sunlight.

Methods: We recruited groups of healthy, nickel-allergic female volunteers. Using a filtered xenon arc lamp, we exposed discrete areas of skin on the lower back of volunteers to graded doses of narrowband UVR. Adjacent, unirradiated areas of skin served as immunologically intact control sites. Following UVR exposure, nickel patches were placed on each of the irradiated and unirradiated sites. The intensity of nickel contact hypersensitivity responses at each site was then measured with a reflectance erythema meter as nickel-induced erythema (NIE) 72 hours later. The degree of immunosuppression was calculated by comparison of the NIE of irradiated sites to the NIE of control unirradiated sites.

Results: The UVB wavelengths 290-310nm caused significant dose-responsive immunosuppression at suberythemal doses. The UVA wavelengths 360-380nm were also immunosuppressive at exposures equivalent to only 15 minutes of Autumn midday sun in Sydney, with loss of the effect at higher exposures.

Conclusions: There are two peaks in the action spectrum for immunosuppression in humans with fundamental differences in the dose-response characteristics between these peaks. UVA is approximately 20 times more abundant in solar radiation than UVB and therefore the solar effectiveness for immunosuppression is greater for UVA than UVB. The results highlight the need for sunscreen protection against long-wave UVA.

OC220

Topically applied vitamin D activates regulatory T cells in naive mice

Shelley Gorman, Alexandra Kuritzky, Melinda Judge, Prue Hart

Telethon Institute for Child Health Research, Perth, Western Australia, Australia

The purpose of this study was to determine whether the active form of vitamin D (1,25-dihydroxyvitamin D3 or 1,25[OH]2vitD3) induces regulatory T cells in topically treated mice. We compared the effect of topical 1,25[OH]2vitD3 with that of ultraviolet B (UVB) irradiation, the environmental factor that is required for the production of 1,25[OH]2vitD3 in skin. Adult female BALB/c or ovabumin T cell receptor (OVA TCR) trangenic mice (DO11.10) were exposed to 125 ng 1,25[OH]2vitD3, vehicle or 8 kJ/m^2 UVB by topical application to shaved dorsal skin. Four days post-treatment, the draining lymph nodes (DLN) were removed and CD4+ T cells purified using standard magnetic bead techniques. These CD4+ T cells were adoptively transferred into naïve BALB/c recipients. Adoptive transfer of CD4+ T cells from the DLN of 1,25[OH]2vitD3-treated or UVB-irradiated BALB/c mice significantly suppressed contact hypersensitivity (CHS) ear swelling responses in recipient mice. Depletion of CD25+ cells prior to adoptive transfer prevented this suppression. Furthermore, the transfer of CD4+CD25+ T cells from the DLN of 1,25[OH]2vitD3-treated or UVB-irradiated mice significantly suppressed ear-swelling responses. In a different model, CD4+ T cells from the DLN of 1,25[OH]2vitD3- or vehicle-treated DO11.10 mice were adoptively transferred into naive recipients. These recipient mice were sensitised 24 h later with OVA (20 µg) in an Aluminium hydroxide adjuvant. Three days later, in the DLN there was a significant increase in the proportion of CD4+ T cells of recipient origin expressing Foxp3, a well-described regulatory T cell marker. Thus, topical 1,25[OH]2vitD3 activates regulatory T cells in the DLN of treated mice. These data indicate that the synthesis of 1,25[OH]2vitD3 in skin following UVB irradiation may be responsible for the activation of regulatory T cells in the lymph nodes of UVB-irradiated mice.

OC221

The effect of UV on effector and memory T cells

Sabita Rana, Scott N Byrne, Linda J Macdonald, Gary M Halliday

University of Sydney, Sydney, NSW, Australia

Exposing C57BL/6 mice to low dose ultraviolet-B (UVB) radiation systemically suppresses the immune system to decrease primary and secondary contact hypersensitivity responses, which are regulated by T cells. However, the effects of UVB on *in vivo* T cell responses during UVB-induced immunosuppression are unknown. In this study, we show that exposure of mice to low dose UVB prior to contact sensitization, inhibits the expansion of effector $CD4^+$ and $CD8^+$ T cells in skin-draining lymph nodes and it reduces the migration of leukocytes, including $CD4^+$ and IFN-γ^+ $CD8^+$ T cells into challenged ear skin. In the absence of UVB and at 10 wk post initial hapten exposure, sensitized mice were infiltrated by dermal effector memory $CD8^+$ T cells to the ear skin at the site of

challenge. However, if mice were previously exposed to UVB, this population was absent suggesting an impaired development of peripheral memory T cells. These data indicate that *in vivo* T cell responses are prone to immunoregulation by UV, including a novel effect of UV on the size of the activated T cell pool, and the development of memory T cells in peripheral compartments.

OC222

Bathochromically shifted hypericin derivatives: photosensitizing properties

Mieke Roelants[1], Heinz Falk[2], Bernd Lackner[2], Mario Waser[2], Peter A.M. de Witte[1]

[1]KU Leuven, Leuven, Vlaams-Brabant, Belgium; [2]Institute of Organic Chemistry, Johannes Kepler University, Linz, Austria

Hypericin, a hydroxylated phenanthroperylenequinone present in a number of plants of the genus Hypericum,exhibits a high fluorescence and localizes very selectively in superficial urothelial carcinomas and carcinoma in situ (CIS) following intravesical administration of the compound in patients. Since hypericin is also a potent photosensitizer, its specific localization in these malignant bladder lesions offers an unique opportunity not only to detect, but also to treat the tumor lesions photodynamically. The light absorption maximum of hypericin (592-595 nm) however somewhat limits its PDT prospect, as non-superficial bladder tumors cannot be treated adequately. Hence, seven hypericin derivatives with an expected bathochromic shift were synthesized.We examined the photosensitizing properties of these compounds, more specifically their photocytotoxic effect on RT-112 cells and their singlet oxygen yield. The outcome shows that there is a correlation between the average cellular accumulation and the photsensitizing properties of the hypericin derivatives. As, compared to hypericin, some of these compounds combine a bathochromic shift with an enhanced hydrophilic character, these derivatives have major prospects for clinical PDT setting.

OC223

Photosensitised reactions by photoinitiators used for curing dental polymer materials

Terje Christensen[1,2], Else Morisbak[1], Inger Sofie Dragland[1], Hanne Hjorth Tønnesen[3], Ellen M. Bruzell[1]

[1]Nordic Institute of Dental Materials, Haslum, Norway; [2]Norwegian Radiation Protection Authority, Østerås, Norway; [3]School of Pharmacy, University of Oslo, Oslo, Norway

Light cured dental polymer materials are today frequently used in dentistry, and the use of amalgam as filling material is decreasing. Amalgam contains mercury which could represent a health hazard. The toxicity and phototoxicity of the new class of materials are not completely investigated. It has been shown that certain monomers produce reactive oxygen species (ROS) without light and that compounds used as photoiniators for polymerisation of these monomers produce ROS upon irradiation with UVA-radiation and blue light. Two photoinitiators were tested for photosensitising activity on rat submandibular gland acinar (oral) cells in culture: Camphorquinone (CQ), and 1-phenyl-1,2-propanedion (PPD). CQ absorbs mainly blue light while the absorption of PPD also includes radiation in the UVA. The cells were irradiated in a photopolymerisation chamber, Polylux PT, equipped with two compact fluorescent tubes emitting in the UVA (Ralutec 9W/78) and one emitting blue light (Ralutec 9W/71). The irradiance was 3.0 mW/cm^2 in the UVA and 7.5 mW/cm^2 in the blue, respectively. Information on cell toxicity was obtained by staining the cells with fluorescent probes, the amount of hydrogen peroxide was determined by a technique reading fluorescence on microplates and singlet oxygen production was assayed by measuring luminescence in the infrared. PPD, in contrast to CQ, produced large amounts of hydrogen peroxide during irradiation, while no singlet oxygen was found during irradiation of any of the compounds. Dark toxicity was not observed for any of the concentrations used. Exposure of cells to the combination of photoinitiators and irrradiation caused reduction in cell number and inhibition of cell multiplication. Relatively more apoptosis and less necrosis were observed in cell cultures irradiated in the presence of CQ compared with PPD. Cell death increased with light dose in the presence of the photoinitiators. Radiation from the light source caused significant cytotoxic effects in absence of photoinitiators. No definite conclusion can be drawn about reciprocity between concentration and irradiation time, but a tendency was seen of increased photobiological effects for high concentrations of CQ. It can be concluded that dental material photoinitiators may have prominent photosensitising effects, and that different initiators act by different photochemical and cell biological mechanisms.

OC224

Photoactivated therapeutic metal complexes

Fiona S Mackay[1], Julie A Woods[2], Patrick J Bednarski[3], Pavla Heringova[4], Jana Kasparkova[4], Viktor Brabec[4], Harry Moseley[2], James Ferguson[2], Peter J Sadler[1]

[1]University of Edinburgh, Edinburgh, Scotland; [2]University of Dundee, Dundee, Scotland; [3]University of Greiswald, Greiswald, Germany; [4]Academy of Sciences of the Czech Republic, Brno, Czech Republic

We have synthesised a series of Pt(IV) prodrugs that are activated to a cytotoxic species when irradiated with light. The activated Pt(IV) complexes rapidly bind to and crosslink nuclear DNA, quickly killing tumour cells including ovarian A2780 carcinoma cells, 5637 bladder cancer cells, and their cisplatin-resistant derivative lines. One of the complexes described has an IC_{50} value 50 fold lower than cisplatin tested under the same experimental conditions. Interestingly, the most photoactive compounds possess the *trans* configuration. Cell-free experiments have characterised the adducts formed (mainly DNA-DNA and DNA-protein crosslinks) and established that their repair is less effective than those formed by cisplatin. The spectrum of lesions formed on photoactivation, and the decreased repair efficacy may explain why these complexes have equal or greater potency than cisplatin under the same conditions. Cell morphology studies and measurement of caspase activity by activation assay and western blotting show important differences between the parent compound and cisplatin. Thus, although the activated complexes have some features in common with cisplatin, other aspects of their mechanism are novel and therefore these complexes may represent a new class of Pt anticancer drug and are not simply acting as prodrugs of cis- or transplatin. The prodrugs have no or limited toxicity in the dark, and do not interact with DNA in the absence of irradiation. Despite their success, traditional Pt-therapeutics have significant dose-limiting side effects, and tumours can acquire resistance. Pt(IV) complexes have anti-tumour properties and can overcome resistance. The photoactivated Pt(IV) complexes described here harness the potent cytotoxic properties of Pt-drugs, but because they are selectively activated by light *only* at the target site they promise a number of clinical benefits.

OC225

Effect of aggregation on photolysis of Merocyanine 540

Elena A. Kozhinova, Andrei M. Tikhomirov, Alla A. Kyagova, Alexander Ya. Potapenko

Russian State Medical University, Moscow, Russian Federation

Merocyanine 540 (MC540) is an anionic dye used as a fluorescent probe and photodynamic photosensitiser. Products of MC540 photooxidation can induce apoptosis in tumor cells and modulate T cell immunity in mice. In this presentation, effect of KCl and phosphate buffered saline (PBS) on aggregation and photolysis of MC540 was studied. It is known that in pure water, the MC540 absorption spectrum exhibits two bands at 500 and 535 nm

attributed to dimer and monomer forms of the dye. On addition of small amounts of salts of monovalent cations hypochromic effect is initially observed without changes in the shape of absorption spectra. Above critical salt concentration (CSC) maxima at 500 and 535 nm gradually disappear and a new absorption band at 517 nm appears. The CSC value was estimated as inflection point on the dependence of optical density at 517 nm on salt concentration. We found hyperbolic dependence of CSC on the dye concentration: CSC=*b*/[MC540], where *b* is a constant. The value of CSC comprised *ca*. 0.12 and 0.05 M KCl at 10 and 20 μM of MC540, respectively. To elucidate the mechanism of salt-induced aggregation we measured Resonance Light Scattering (RLS) spectra. The RLS effect is observed as increased Rayleigh scattering intensity at or very near the wavelength of absorption of an aggregated molecular species when strong electronic coupling exists among the chromophores. RLS spectra were registered with a fluorimeter in the synchronous scanning regime of the excitation and emission monochromators in the range from 220 to 640 nm, and then corrected. MC540 in pure water did not exhibit any RLS signal. Addition of salts below CSC led to appearance of broad RLS band at 420-460 nm belonging to extended H-aggregates of the dye. A new intensive RLS band (very similar to absorption band) appeared at about 517 nm above the CSC. Photochemical experiments have shown that photochemical lability of different aggregation forms of MC540 decreased in a row: extended supramolecular aggregates (revealed by RLS) >> dimers >> monomers.

Acknowledgement: This research was supported by the RFBR Grants #04-04-49626a, 07-04-01060a.

OC226

Influence of lycopene on UVR-induced erythema and mitochondrial DNA damage in human skin

Muneeza Rizwan[1], Andrew Harbottle[2], Isabel Rodriguez-Blanco[1], Rachel Watson[3], Mark Birch-Machin[2], Lesley Elisabeth Rhodes[1]

[1]Photobiology Unit, Dermatological Sciences, University of Manchester, Manchester, United Kingdom; [2]Dermatological Sciences, Medical School, University of Newcastle Upon Tyne, Newcastle Upon Tyne, United Kingdom; [3]Dermatological Sciences, University of Manchester, Manchester, United Kingdom

Lycopene is a carotenoid antioxidant found in tomatoes and other red fruits and vegetables, with anti-carcinogenic properties. We have examined its potential photoprotective properties in (i) UVR-induced skin erythema, by use of an erythema dose-response model, and (ii) UVR-induced mitochondrial DNA damage, a biological dosimeter for UVR exposure.

A randomised controlled dietary supplementation trial was performed in 20 healthy females (mean age 33 yrs, range 21–47 yrs; all skin type I/II) who received either 55g tomato paste (containing 16 mg lycopene) in olive oil, or olive oil alone, daily for 12 weeks. At the beginning and end of the supplementation period, a geometric series of 10 UVR doses were applied to buttock skin using a broadband UVB lamp, and the erythemal response was assessed at 24 hours, both visually, as the minimal erythema dose (MED), and objectively through quantification with a reflectance instrument. Biopsies were taken from unexposed and 3xMED UVR-exposed skin at the beginning and end of supplementation, and were assessed for mitochondrial DNA damage using qPCR assay for analysis of UVR-induction of the Mt 3895 deletion (Harbottle & Birch-Machin. *Br J Cancer* 2006, 94, 1887-93).

The median post supplement MEDs in the tomato paste and control groups were 38 (range 23-53) vs. 27 (range 15-53) mJ/cm^2 respectively, p=0.087. The mean post supplement D30, determined quantitatively by erythema dose response modelling, was 36.6 (SD 14.7) vs. 23.0 (SD 6.6) mJ/cm^2 in the tomato paste and olive oil groups respectively, p=0.028. UVR-induced cutaneous mitochondrial DNA damage was reduced after tomato paste, p<0.05, but showed no significant change after control supplement.

This pilot study indicates protection by lycopene against acute UVR-induced skin effects. Further, it illustrates that the relatively crude assessment of the visual MED may fail to detect protection evident on dose-response modelling and tissue analysis. Small degrees of UVR-protection obtained from lycopene-containing food products could over a longer period play a significant role in promotion of skin health.

This research was supported by the British Broadcasting Corporation (BBC2).

OC227

Fluorescence dynamics of UV-B sunscreens

Thomas M. Nordlund[1], Rajagopal Krishnan[2]

[1]University of Alabama at Birmingham, Birmingham, Alabama, United States; [2]University of California at San Francisco, San Francisco, California, United States

We describe the excited-state relaxation of three UV-B sunscreens by time-resolved fluorescence and steady-state quantum yields. The results suggest that octyl methoxy cinnamate and padimate O have low radiative rate “dark” states, in addition to the normal excited states; octyl salicylate does not. The radiative rates of the dark states are of order 10^6 s^{-1}. “Dark” states are indicated when high extinction coefficient sunscreens, which have correspondingly high theoretical (Strickler-Berg) radiative rates, do not show lifetimes and total emission consistent with such a high radiative rate. Note that a high radiative rate, accompanied by efficient fluorescence emission, may be unfavorable for a sunscreen, depending on what effects the emitted light has. We have shown in one case that such sunscreen emission can excite collagen in skin.

Understanding excited state processes in a mixed polarity model system similar to skin is essential. We report the excited state lifetimes, quantum yields, radiative and non-radiative rates of three sunscreens in several solvents and adsorbed to polystyrene microspheres suspended in aqueous buffer. Octyl salicylate emits from a single excited state, while padimate O and octyl methoxy cinnamate show multiple states in solvents. The radiative rates of octyl salicylate and octyl methoxy cinnamate are almost independent of solvent, while that of padimate O varies strongly. The non-radiative rates of all three sunscreens vary with dielectric constant. Compared to octyl salicylate and octyl methoxy cinnamate, padimate O is complex to analyze because of its two emission bands and one band’s strong dependence on the solvent. High absorbance, a broad absorption peak with small fluorescence quantum yield and radiative rate give octyl methoxy cinnamate some superior UV-B photophysical properties compared to those of octyl salicylate and padimate O.

The complexity in the analysis of fluorescence decay and quantum yield suggests that the lifetimes of the sunscreens are critical parameters to determine, in addition to the theoretical radiative rates and the quantum yields. Fluorescence lifetime supports the use of polystyrene nanospheres as a model host to study the photo-physical properties of sunscreen in a heterogeneous environment.

OC228

***In vivo* relevance for photoprotection by the vitamin D rapid response pathway**

Katie M Dixon[1], Shivashni S Deo[1], Anthony W Norman[2], June E Bishop[2], Gary M Halliday[3], Vivienne E Reeve[4], Rebecca S Mason[1]

[1]Department of Physiology and Bosch Institute, The University of Sydney, Sydney, NSW, Australia; [2]Department of Biochemistry, University of California, Riverside, CA, USA; [3]Department of Medicine (Dermatology), The University of Sydney, Sydney, NSW, Australia; [4]Faculty of Veterinary Science, The University of Sydney, Sydney, NSW, Australia

Vitamin D is produced in skin following exposure of its precursor, 7-dehydrocholesterol, to ultraviolet irradiation (UVR). It is

converted in skin to the biologically active metabolite, 1,25-dihydroxyvitamin D3 [$1,25(OH)_2D_3$], which can exert its functions via two distinct pathways: a classical steroid receptor/genomic pathway, or a rapid non-genomic pathway mediated by a putative membrane receptor. Whether the rapid response pathway is physiologically relevant is unclear. We previously reported that $1,25(OH)_2D_3$ at picomolar concentrations, protects human skin cells from UVR-induced apoptosis, and decreases mutagenic DNA photolesions in the form of cyclobutane pyrimidine dimers (CPD) in surviving cells. A *cis*-locked, rapid-acting agonist $1,25(OH)_2$lumisterol$_3$ (JN), entirely mimicked the actions of $1,25(OH)_2D_3$ to increase p53 expression and reduce keratinocyte loss and CPD damage after UVR. The effects of $1,25(OH)_2D_3$ were abolished by a rapid-acting antagonist, whilst a genomic antagonist had no effect. Skh:hr1 mice exposed to 3 MED of solar-simulated UVR and treated topically with $1,25(OH)_2D_3$ or JN immediately after UVR showed reduction in UVR-induced oedema and erythema compared with vehicle-treated mice. Sunburn cells were reduced 24h after UVR in mice treated with $1,25(OH)_2D_3$ or JN from 2.7 ± 0.4 to 1.0 ± 0.3 ($p<0.01$) and 1.2 ± 0.1 ($p<0.01$) per linear mm respectively. Topical $1,25(OH)_2D_3$ or JN at similar doses reduced CPD measured 24h post UVR from $9 \pm 3\%$ to $3 \pm 1\%$ ($p<0.01$) and $4 \pm 2\%$ ($p<0.01$) respectively. Topical $1,25(OH)_2D_3$ and JN significantly reduced systemic UVR-induced immunosuppression in Skh:hr1 mice from $23 \pm 1\%$ to $5 \pm 1\%$ ($p<0.001$) and $-3 \pm 2\%$ ($p<0.001$) respectively. A photocarcinogenesis model in which mice were exposed to chronic low dose UVR and treated topically immediately following UVR resulted in significant reductions in skin tumor formation in mice treated with $1,25(OH)_2D_3$ and JN compared with vehicle-treated mice. These results show for the first time an *in vivo* biological response mediated by a rapid-acting analog of the vitamin D system and support the hypothesis that $1,25(OH)_2D_3$ exerts its photoprotective effects via the rapid pathway. Furthermore, the data suggest a role for vitamin D compounds as skin cancer preventative agents.

OC229

Is the seasonal variation of cancer prognosis related to photosynthesis of vitamin D?

Alina Carmen Porojnicu[1], Johan Moan[1,2]

[1]Rikshospitalet Radiumhospitalet HF, Oslo, Norway; [2]Department of Physics, University of Oslo, Oslo, Norway

The sun is the most important source of vitamin D for humans. Sun beds can also provide substantial amounts of vitamin D. Even so-called UVA sun beds are good sources of vitamin D, since they emit some UVB which is orders of magnitude more efficient than UVA both with respect to vitamin D photosynthesis and melanogenesis. UVB exposure converts 7 dehydrocholesterol (7-DHC) in skin to previtamin D_3, which isomerizes to vitamin D_3. Vitamin D_3 binds to D-binding protein, and is transported to the liver, where hydroxylation to 25-hydroxyvitamin D_3 (calcidiol) takes place. Calcidiol is present in human serum in concentrations of 10 – 100 nmol/L, and is regarded to be a reliable measure of the vitamin D status. Calcidiol is further hydroxylated to 1,25-dihydroxyvitamin D_3 (calcitriol) in the kidney. Calcitriol is believed to be the active hormone at least with respect to bone metabolism. In view of cancer protection, however, our work indicates that calcidiol may be more important. Many tumors have receptors for calcidiol. We find that the calcidiol level is 20-50% higher in late summer than in late winter, and we believe that this may explain the improved survival of a number of cancer forms if treatment is started in the season of high calcidiol levels.

Although rickets is prevented at a serum level above 12.5 nmol/L, the general optimal level is somewhere between 100 and 250 nmol/L. To reach such a level a daily intake of 100-200 µg vitamin D may be required. This equals about 50-100 ml cod liver oil. Sun or sun bed exposures amounting to about two minimal erythema doses (MEDs) per week would give the same calcidiol level. One MED is the UV exposure that gives a slight erythema (skin redness) and is achieved in about 10-20 minutes at midday in midsummer in the Nordic countries.

An adequate vitamin D level counteracts many cancer forms (prostate-, breast-, colon-, lung-cancer as well as lymphomas). Furthermore, incidence rates, or severity, of several other diseases are decreased by sufficient vitamin D levels: multiple sclerosis, diabetes, rheumatoid arthritis, multiple sclerosis, osteomalacia and cardiovascular diseases. The action mechanisms of vitamin D may be related to its immune- and cell-differentiating effects. Most of the 250 cases of death of skin cancer in Norway are due to sun exposure. Nevertheless, one should consider the health advantages of carefully increased overall sun or sun bed exposure, notably for old persons and immigrants with dark skin and for persons who rarely expose their skin to solar radiation.

OC230

Circadian effectiveness of solar and artificial radiation in dependence on age

Helmut Piazena[1], Leonora Franke[2], David Sülflow[2], Katharina Stark[2], Dieter Kockott[3], Ralf Uebelhack[2]

[1]Klinik für Psychiatrie und Psychotherapie der Charité, Berlin, Berlin, Germany; [2]Charité, Berlin, Berlin, Germany; [3]Dr. D. Kockott UV-Technik, Hanau, Germany

The aim of the investigation was to determine the threshold irradiance of sufficient melatonin suppression for persons of different age with free pupil adaptation by using polychromatic radiation with different emission spectra and with different angles of incidence on the cornea. These data were used to evaluate the circadian effectiveness of solar irradiance at the Earth´s surface and of different lamp types for children, young adults and seniors. Melatonin suppression was measured directly by analysing blood samples of volunteers classified by their age and exposed by polychromatic radiation in dependence on its spectral distribution, on its irradiance and on its incidence angle on the cornea. Effective threshold irradiances to get saturation of melatonin suppression were calculated by using the circadian action spectrum. Depending on the age, the data ranged between about 0.2 $W\ m^{-2}$ and about 0.6 $W\ m^{-2}$ in the case of half spheric geometry and of different emission spectra of white light lamps. In contrast, decreases of the incident angle resulted in decreases of melatonin suppression even if the luminance was increased to get equivalent corneal irradiance. However, the threshold irradiances experimentally determined for persons of different age are approximately in line with the thresholds calculated by extrapolation by using age-dependent spectra of eye transmittance and threshold irradiance data of melatonin suppression measured in young adults with dilated pupils and with monochromatic radiation. This result is discussed as reference to the applicability of the additivity law of photobiology to evaluate circadian effectiveness of polychromatic light sources by weighting with the circadian action spectrum, whereas the experimental data clearly show the violation of the *Bunsen-Roscoe* law as well as the need to establish “circadian weighted” measures to exclude confusion by using measures of visual effectiveness which result in misinterpretation of circadian effectiveness. In addition, ageing effects of the eyes have to be considered to evaluate circadian effectiveness and effects of light. Outdoor sun light exposures during cloudless sky cause sufficient melatonin suppression between sunrise and sunset for persons of all ages, whereas the suitable daily periods are limited in case of cloud covered sky and depend on latitude, season, age, type of cloudiness and degree of cover. Lamps show different ratios between circadian and visual effectiveness in dependence on a person´s age which may be used to stimulate or to prevent melatonin suppression.

IL231

Recent developments in the application of PAM chlorophyll fluorometry

Wolfgang Bilger
University of Kiel, Kiel, Germany

The photosynthetic apparatus is sensitive towards environmental stresses. Therefore, it may serve as a bioindicator, provided its state can be easily and rapidly detected. This is the case with techniques applying chlorophyll fluorescence. Over the last decades various fluorometers have been developed employing the technique of Pulse Amplitude Modulated (PAM) fluorometry for a wide variety of purposes. Recently, the technique has been further refined with the advent of powerful strong LEDs, allowing high spatial and temporal resolution of fluorescence signatures. Results will be presented on the influence of stress using different types of new apparatus. A fluorescence imaging system will used to demonstrate spatial heterogeneity within single leaves and whole plants. A dual excitation system will be presented to determine electron transport through both photosystems, I and II, at the same time. This system can also be used to differentiate inhibition at the donor and the acceptor side of photosystem II. Potentials and limitations of the method will be discussed.

IL232

Phytoremediation of metal polluted soils: a sunlight-driven environmentally friendly technique

Franco Gambale, Monica Bregante
Istituto di Biofisica, CNR, Genova, Italy

Metal contamination is a problem throughout Europe. The cleanup and remediation of metal-contaminated soils is notoriously difficult. However, the risks to humans posed by high concentrations of these contaminants means that there is a pressing need for soil remediation.

Many current technologies for soil remediation imply high application costs, and excavation of the contaminated soil. These methods are often not cost effective on the basis of the returns that can be realistically generated from post-remediated sites.

Phytoremediation has recently aroused considerable interest as an in-situ, light-driven plant-based soil cleanup method. Of the various phytoremediation methods (phytostabilisation, phytoextraction, rhizofiltration, phytodegradation), phytoextraction appears to be economically the most advantageous. Phytoextraction is based on the principle that plants can be compared to solar energy-powered pumps capable of extracting metals from the soil and transferring them to aerial organs. There are essentially two approaches to phytoextraction, continuous and assisted phytoextraction. Results obtained within a pilot project, using assisted phytoremediation to clean lead polluted soils, are illustrated.

IL233

Treatment of microbiologically polluted aquaculture waters by a novel photochemical technique of potentially low environmental impact

Michela Magaraggia[1], Filippo Faccenda[2], Fabiola Paterno[1,2], Andrea Gandolfi[2], Giulio Jori[1]
[1]University of Padova, Department of Biology, Padova, Italy; [2]IASMA, Research Center, Limnology and Fish Research Unit, Natural Resources Department, San Michele a/A, Trento, Italy

Saprolegniasis is an infection of freshwater fish and eggs caused by the water mold *Saprolegnia* spp. and causes large economic losses in aquaculture. Formalin is the only fungicide used in aquaculture that could replace malachite green, which is currently banned owing to its carcinogenic potential. However, there are concerns about the possible toxic effects of formalin on the environment, as well as on fish and consumer health. Therefore, the search for alternative fungicides is attracting increasing attention. The present study aims at investigating the potential of porphyrin-type photosensitizers to combat saprolegniasis using (a) cell cultures of a fungal pathogen (*Saprolegnia* spp.); (b) pilot aquaculture plants involving either spontaneously or artificially *Saprolegnia*-infected rainbow trout (*Oncorhynchus mykiss*) and trout eggs. Toward this aim, we selected two cationic porphyrins, i,e. a tetra(N-methyl-pyridyl)porphine (C1) and its analogue where one N-methyl group has been replaced by a N-tetradecyl chain (C14), which proved to be very efficient photocydal agents against both Gram-positive and Gram-negative bacteria, including antobiotic-resistant strains. Thus, exposure of *Saprolegnia* cells to low intensity visible light induced an extensive (up to 6-7 log) decrease in the fungal population after short incubation and irradiation times in the presence of micromolar photosensitiser concentrations. Extension of these studies to the pilot plants indicated that both C1 and C14 + light can prevent *Saprolegnia* infections or promote the complete remission of saprolegniasis in infected trout by treatments with submicromolar porphyrin doses. C14 showed some cytotoxicity even in the dark. Moreover, the pre-irradiation of water to be used for incubation of trout eggs with visible light in the presence of 0.5 mg/l C14 appeared to bring about a very efficient protection against *Saprolegnia* infections with no detectable effect on embryo morphology and physiology. The procedure appears to be of low cost and to have a low environmental impact.

IL234

Automatic online bioassay system to monitor aquatic ecosystems using movement responses in unicellular microorganisms

Donat-Peter Häder
Friedrich-Alexander Universität, Institut für Botanik, Erlangen, Germany

Bioassays are useful systems to determine pollutants and toxins in aquatic ecosystems. Fish, crustaceans and algae have been used in the past to detect potentially dangerous levels of chemicals in drinking water, waste water and natural water bodies. An automated fast biomonitoring system for early warning of pollutants in aquatic environments has been developed. The system uses changes in the movement behavior of unicellular flagellates as biological endpoints determined by real time image analysis. All parameters describing motility, velocity, orientation with respect to light and gravity and form of the cells are calculated during measurement, and changes of these parameters are quantified. By automatic dilution of the water sample dose-effect relationships can be determined automatically. A total measurement procedure including control and sample measurement, filling and rinsing of the system typically requires 5 min. The system was tested with different organic and inorganic toxic compounds and the calculated EC50 values were compared with literature data for other bioassays. Measurements with industrial and household waste water samples were also performed. The fast response time, the small size, the reliable image analysis system, the calculation of several endpoints and the automatic measuring procedure are major advantages compared to other biological test systems.

IL235

Testing the presence of tributyl-Sn in marine water using microorganism behaviour

Nicola Messina[1], Enrico De Gubernatis[1,2], Fernando Dini[2], Francesco Ghetti[1], Giovanni Checcucci[1]
[1]Istituto di Biofisica CNR, Pisa, Italy; [2]Dipartimento di etologia, ecologia ed evoluzione, Università di Pisa, Pisa, Italy

Unicellular protists are widespread organisms populating every ecosystem. They include both generalist and specialist species and constitute an extremely important part of the food chains. They are therefore suitable model organisms to be used as biomonitors. In fact, as unicellular protists are at the same time "cell" and

"organism", both their physiological and behavioural responses can constitute indicators of the presence of environmental pollutants.
We present preliminary data about the effect of tributyl tin, a well known marine pollutant which is used in anti-fouling paints for ship hulls, on photosynthesis and behaviour of selected protists. The investigation have been carried out by means of PAM fluorometry and computer-assisted track analysis, monitoring optimal photosynthetic quantum yield, motility and photoresponse. The selected microorganisms were the green alga *Dunaliella salina*, the diatom *Phaeodactylum tricornutum*, the dinoflagellate *Oxyrrhis marina* and the ciliate *Fabrea salina*. These microorganisms showed different sensitivity to tributyl tin. The photosynthetic efficiency of *P. tricornutum* was unaffected, whereas *D. salina* showed a decrease in photosynthetic efficiency at concentrations up to about 10^{-7} M. A similar result was observed in *D. salina* as far as motility is concerned. Promising results have been obtained investigating the behaviour of *O. marina*.

OC236

Insecticidal effects of phloxine-B on *Bactrocera zonata* and its symbiotic bacteria

Amira Abdou AlAdly
NILES , Cairo University, Cairo, Egypt

The insecticidal properties of the photosensitizer phloxine-B (PH-B) was evaluated against Peach fruit fly, *Bactrocera Zonata* and its symbiotic bacteria in laboratory bioassays. Groups of *B. zonata* flies were fed on agarose-based sugar media containing range of concentrations of the photosensitizer for 24 hours in dark, and then exposed to solar simulator light at fluence rate of 400 W/m^2 for 2 hours at 25 ± 1ºC.
It has been found that the photosensitizer had no detectable lethal effect on adults of *B. zonata* and its symbiotic bacteria in the absence of light. Under the influence of light, high rates of mortality were achieved among flies fed on PH-B, with LC50 values of 0.0024%. The degree of toxicity of PH-B against *B. zonata* adults was affected by fluence rate, temperature, duration of exposure and time after exposure.

Keywords: *Bactrocera zonata*, Peach fruit fly, phloxine-B, *Klebsiella pneumoniae.*

OC237

Photochemical transformation of dissolved organic matter in natural humic waters and humic isolate solutions

Sarka Klementova[1], *Petr Porcal*[2], *Jiri Kopacek*[2], *Dalibor Kriz*[1]
[1]*Fac. of Biol. Sci., Univ. South Bohemia, Ceske Budejovice, Czech Rep.;* [2]*Inst. of Hydrobiology, Academy of Sci. Czech Rep., Ceske Budejovice, Czech Rep.*

The laboratory photochemical experiments with natural organic matter were carried out with natural humic waters and artificially prepared humic solutions. The natural humic waters came from water bodies and there inlets in the region of the Bohemian Forest, Czech Rep. Artificial humic solutions were prepared from humic and fulvic acids samples isolated from different soil horizons in the same region. Irradiaion of the samples with UV light (300-400 nm, maximum emission at 350 nm) was performed for up to 48 hours. The study was focused on changes in spectroscopic characteristics of organic matter during irradiation (fluorophores identification), on photochemically induced hydroxyradical formation (measured by eriogalucine bleaching) and its influence on spectroscopic characteristics, and also on changes in metals speciations.

Financal support of Grant agency of Czech Republic (grant No. 206/06/0410) is gratefully acknowledged.

IL238

Antimicrobial PDT: exploiting target cell function for selective photosensitizer delivery

Tayyaba Hasan, Sarika Verma, Xiang Zheng, Ulysses Sallum, Humra Athar
Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School, Boston, USA

Photodynamic therapy (PDT) is emerging as a potentially useful alternative antimicrobial therapy, especially in the treatment of drug-resistant localized infections. As the application of PDT expands with regards to both anatomical sites and disease stages, it is increasingly becoming important to develop strategies for enhancing PDT outcomes.
Specificity in PDT arises due to the site-directed generation of cytotoxic effects upon targeted light administration to activate photosensitive chemical compounds (PS). The specificity can further be improved by not only targeting a PS to a specific site of action, but also by creating an active form of a PS from an inactive (quenched) form by using specific cellular functions (such as enzymes) at the site of action. In this case, an inactive PS is administered such that it is activated (and produces cytotoxic effects) only at the site of lesion. This method has been utilized for both cancer and anti-microbial PDT. Although it is difficult to specifically demarcate between cancerous cells and non-cancerous cell markers, functional-sensitive linkages have been significantly beneficial for picking specific markers for infectious diseases such as microbial infections, where enzymatic expressions are clearly differentiated from the host environment. The enzymatic compositions of bacterial and mammalian cells are significantly dissimilar, offering an opportunity for capitalizing the difference for achieving high selectivity against bacterial cells. Our group is exploring various strategies for exploiting functional molecular targets to achieve selective PDT. Initial data and implications from our early experiments will be discussed.

IL239

Targeting proteolytic activity: new opportunities in fluorescence diagnosis and photodynamic therapy

Norbert Lange
Department of Pharmaceutics and Biopharmaceutics, School of Pharmaceutical Sciences, University of Geneva - University of Lausanne, Quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland

Peptidases and proteases play an essential role in the establishment and progression of many diseases, including cancer, rheumatoid arthritis, arteriosclerosis and bacterial infections. Since the early 70's quenched fluorogenic substrates have been designed to mach the specificity of a particular proteolytic enzyme. In simple designs of such substrates a fluorophore is coupled via protease specific peptide linker to a fluorescence quencher. Enzymatic cleavage of this linker suppressed the quenching effect and an increase of fluorescence indicates the proteolytic activity of the targeted enzyme. In principle such constructs are appropriate for the fluorescence diagnosis of proteolytic activity in vivo.
However, more recently the group of Ralph Weissleder at MGH in Boston has exploited a new concept in which multiple photoactive moieties are coupled either directly or via a peptide linker to a polymeric backbone, thus, resulting in a self quenching of fluorescence. Again, enzymatic digestion then restores the fluorescence which can be easily monitored by fluorescence imaging devices in vitro and in vivo.
By coupling compounds with a relatively high singlet oxygen quantum yield to such protease sensitive constructs, these concepts can also be exploited for the site specific delivery of photosensitizers in photodynamic therapy.
Recently, several research groups have followed these approaches and the present review will summarize the efforts and outcomes this research.

IL240

Recent improvements in the use of synthetic peptides for a selective photodynamic therapy

Muriel Barberi-Heyob[1], *Noémie Thomas*[1], *Loraine Tirand*[1], *Denise Bechet*[1], *Régis Vanderesse*[2], *François Guillemin*[1], *Céline Frochot*[3]

[1]*Centre Alexis Vautrin-CRAN, UMR 7039 CNRS-UHP-INPL, Nancy University, Vandoeuvre-les-Nancy, France;* [2]*LCPM, Groupe ENSIC-UMR 7568 CNRS-INPL, Nancy University, Nancy, France;* [3]*DCPR-GRAPP, Groupe ENSIC-UMR 7630 CNRS-INPL, Nancy University, Nancy, France*

The selectivity of photodynamic therapy (PDT) relies both upon the targeting of the light delivery and on the preferential uptake of the photosensitizer (PS) by malignant tissue. However, the tumor selectivity of PSs used in clinics is limited as they tend to accumulate in normal tissue. This can be improved by using third generation PSs, which consist of PSs to which a tumor-targeting moiety is attached. Among these tumor targeting molecules, peptides are receiving increased interest in the field of PDT. Actually, the targeted delivery of PSs to defined cells is a major challenge in PDT of cancer. Alterations or increased levels in receptor expression of specific cellular type occur in the diseased tissues. Therefore, PSs can be covalently attached to molecules such as peptides, leading to a receptor-mediated targeting strategy. These active targeting approaches may be particularly useful for anti-vascular PDT. Peptides do not exist in a natural form but can be designed synthetically as novel molecules. In addition, the effective tissue penetration of short peptides, in combination with their selective binding and internalizing capacity by cancer cells, make them interesting candidates for the delivery of therapeutic agents such PSs.

Moreover, it has been shown that the photocytotoxicity of photodynamic drugs could be enhanced by delivering high amounts of a PS into subcellular organelles. The recent progresses in the use of active – targeting strategy with synthetic peptides and the interest of using an active targeting strategy in PDT will be presented.

IL241

Photochemical internalization: a technology for site-specific drug delivery

Kristian Berg[1], *Pål Kristian Selbo*[1], *Anette Bonsted*[1], *Anette Weyergang*[1], *Ole-Jacob Norum*[2], *Qian Peng*[3], *Anders Høgset*[4]

[1]*The Norwegian Radium Hospital, Dept. of Radiation Biology, Oslo, Norway;* [2]*The Norwegian Radium Hospital, Dept. of Radiation Biology/surgical Oncology, Oslo, Norway;* [3]*The Norwegian Radium Hospital, Dept. of Pathology, Oslo, Norway;* [4]*PCI Biotech, Oslo, Norway*

The utilisation of macromolecules in therapy of cancer and other diseases is becoming increasingly relevant. Recent advances in molecular biology and biotechnology have made it possible to improve targeting and design of cytotoxic agents, DNA complexes and other macromolecules for clinical applications. To achieve the expected biological effect of these macromolecules in many cases internalisation to the cell cytosol is crucial. At an intracellular level, the most fundamental obstruction for cytosolic release of the therapeutic molecule is the membrane-barrier of the endocytic vesicles. Photochemical internalisation (PCI) is a novel technology for release of endocytosed macromolecules into the cytosol. The technology is based on the use of photosensitizers located in endocytic vesicles, that upon activation by light induces a release of macromolecules from their compartmentalization in endocytic vesicles. PCI has been shown to potentiate the biological activity of a large variety of macromolecules and other molecules that do not readily penetrate the plasma membrane, including type I ribosome-inactivating proteins (RIPs), gene-encoding plasmids, adenovirus and the chemotherapeuticum bleomycin. PCI has also been shown to enhance the treatment effect of targeted therapeutic macromolecules. The results show that PCI can induce efficient light-directed delivery of macromolecules into the cytosol, indicating that PCI may have a variety of useful applications for site-specific drug delivery, e.g. in gene therapy, vaccination and cancer treatment. Recent advances in the development of PCI towards clinical utilization will be presented.

OC242

Development and characterisation of a protoporphyrin IX-peptide conjugate for use in the photodynamic therapy of cancer

Clare Louise Conway

University of Leeds, Leeds, United Kingdom

Increasing the selective localisation of photosensitiser to tumour tissue is an aim of much current research. Endothelial cells of the angiogenic tumour neovasculature have a significantly different cell surface protein expression profile to quiescent vessels. Notably, integrin $\alpha_v\beta_3$ is significantly upregulated in proliferating endothelial cells and thus is an interesting molecular target. Natural $\alpha_v\beta_3$ ligands are components of the extracellular matrix and all contain a common exposed arginine-glycine-aspartic acid (RGD) binding motif. By conjugating a small targeting peptide to a photosensitiser, we were able to increase the *in vivo* accumulation of the novel compound in tumour tissue compared to the free photosensitiser. The cyclic peptide, cRGDfK (arginine-glycine-aspartic acid-phenylalanine-lysine) was synthesised using solid phase peptide chemistry and conjugated to the photosensitiser, protoporphyrin IX (PpIX). The cyclic RGDfK peptide is known to be relatively specific for the $\alpha_v\beta_3$ integrin and more resistant to proteolytic degradation than its linear counterpart. This study characterises and compares the *in vitro* and *in vivo* properties of the novel conjugate with that of the free photosensitiser. PpIX and cRGDfK were shown to retain their respective photodynamic and integrin binding activity during coupling. The conjugate was shown to be an excellent photosensitiser *in vitro* and *in vivo*. Pharmacokinetic analysis of PpIX:cRGDfK-treated mice demonstrated a significant retention of photosensitiser in tumour tissue with increased tumour:normal tissue ratios.

OC243

Synthesis of folate directed photodynamic therapy agents

Kenneth W. Olsen, *Godfred Boateng*, *Ping Hu*

Department of Chemistry, Loyola University Chicago, Chicago, Illinois, USA

Photodynamic therapy (PDT) involves administration of a photosensitizer followed by the application of a specific wavelength of light to generate activated oxygen molecules that damage tumor cells. One of the drawbacks to current PDT is the limited specificity of PDT agents for cancer cells. Many types of cancer cells express elevated levels of the folate receptor, making this receptor useful in discriminating cancerous cells from normal ones. Folate-conjugated molecules can be introduced into these cells via folate receptor-mediated endocytosis. We have developed novel compounds for labeling these cells by conjugating folate and fluorescein isothiocyanate (FITC) with each other and to hemoglobin. Folic acid - fluorescein isothiocynate conjugate has been synthesized and characterised by HPLC, ^{1}H-NMR, UV-visible and mass spectroscopy. Cellular accumulation and competitive assay of the conjugate have been evaluated using the SH-SY5Y neuroblastoma cell line. After 24 h incubation cellular uptake of the conjugate was about 6-fold higher than that of fluorescein, suggesting an active transport via endocytosis. Cytotoxicity studies using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] showed that more than 90% of the cells survived at a concentration less than 7 μM of the conjugate. We have also made FITC-folate-hemoglobin complexes. Mass spectroscopy studies show that there is more attachment of folate to the β-chains than to

the α-chains. Transport of the FITC-labeled folate-hemoglobin into SH-SY5Y cells was demonstrated by fluorescence microscopy. Uptake of the labeled compound was blocked by exogenous free folate and no uptake of hemoglobin-FITC complex alone was noted. HeLa cells, which are known to express excess folate receptors, take up FITC-folate-hemoglobin in the same way as the SH-SY5Y cells. Uptake of the FITC-folate-hemoglobin by normal cells, however, was very low. The FITC-folate-hemoglobin did not cause cell death as judged by Trypan Blue exclusion studies. A better photoactive dye will be needed for effective PDT. Taken together, these studies suggest that photodynamic compounds could be selectively delivered to the interior of cancerous cells that over-express the folate receptor by coupling a photodynamic compound to folate. The synthesis described here provides the potential delivery of both the oxygen carrying protein and the photoactive dye to selected cells.

OC244

Hypericin-loaded nanoparticles to improve photodetection of micrometastases in ovarian cancer

Magali Zeisser-Labouèbe, Florence Delie, Robert Gurny, Norbert Lange

Laboratory of Pharmaceutics and Biopharmaceutics, School of Pharmaceutical Sciences, University of Geneva - University of Lausanne, Geneva, Switzerland

Ovarian carcinoma is the fourth most frequent cause of cancer-related death in women in the United States. The currently recommended treatment is a combination of surgery followed by chemotherapy. However, ovarian cancer is characterized by the development of peritoneal micrometastases. Micrometastases are hardly detectable by conventional method and are therefore suboptimally treated by standard surgery. As a consequence, up to 50% of treated patients have a relapse and will inevitably die from their disease. Improving the detection rate using fluorescence detection during resection will potentially influence the therapeutic outcome. Hypericin (Hy), a natural compound extracted from Hypericum perforatum is a potentially interesting drug for fluorescence diagnosis in the field of cancer. The medical development of Hy is hampered by its hydrophobicity, restricting its systemic administration. To overcome this problem, 200 to 300 nm biodegradable nanoparticles (NPs) of polylactic acid (PLA) have been prepared. The selectivity of Hy-loaded NPs for tumoural tissues was investigated in vivo on Fischer rats bearing ovarian tumours. NuTu-19 ovarian cancer cells were intraperitoneally injected in female Fischer 344 rats and the tumour development was achieved after 6 weeks. This model is close to the human ovarian cancer showing the formation of a malignant hemorrhagic ascites and a spread of malignant masses and serosal nodules adherent to all intraabdominal organs. Hy, in solution or loaded in NPs, was intravenously administered. The rats were sacrificed after different times and the fluorescence of the peritoneal cavity was assessed by endoscopy. The preliminary results showed a time-dependent fluorescence intensity of the tumoural nodules with both formulations. However, whereas free Hy was distributed in both tumoural and surrounding tissue, the fluorescence associated with Hy-loaded NPs was more specifically observed in tumour nodules. Thus, the incorporation of Hy in nanoparticles improves the selectivity of Hy for the tumoural tissues.

OC245

Photochemical internalisation can enhance the cytotoxicity of bleomycin and saporin in the A431 cell line

Tzu-wen Wang, Stephen G. Bown, Alexander J. MacRobert

University College London, London, UK

Photochemical internalisation (PCI) is a promising new approach for improving drug delivery to cells. Many anticancer agents, such as bleomycin and saporin, have limited efficacy owing to lysosomal sequestration. We investigated whether we could enhance delivery of these drugs to their intracellular target sites, and in turn their therapeutic efficacy, by photochemical internalisation.

Experiments were carried out using A431 human epidermoid carcinoma cells and several photosensitisers were compared: aluminium phthalocyanine disulfonate ($AlPcS_{2a}$) and disulfonated meso-tetraphenylporphine ($TPPS_{2a}$, PCI Biotech) and other porphyrin analogues. Saporin was co-incubated with the photosensitiser whereas bleomycin was added after the photosensitiser was washed off. Cell viability was determined using the MTT assay. Excitation of the phthalocyanine was carried out using a 670 nm laser, and a blue lamp was used for the other sensitisers. The subcellular distributions of the photosensitisers and Alexa488-labelled saporin before and after light exposure were examined using fluorescence microscopy.

Fluorescence imaging demonstrated good intracellular co-localisation of the photosensitisers with Alexa488-labelled saporin corresponding predominantly to a lysosomal distribution. Both $TPPS_{2a}$ and $AlPcS_{2a}$ intracellular distributions were observed to become more diffuse after light exposure. Enhanced cell kill by saporin or bleomycin was observed in combination with the photosensitisers following light exposure. In combination with saporin (1nM) and $TPPS_{2a}$ (0.1 μg/ml), PCI enhanced the cell kill, reducing the cell viability more than 6-fold after 3 min light exposure compared to saporin treatment alone. The cell viability was reduced 21-fold by $AlPcS_{2a}$ (5μg/ml) PCI treatment after 3 min light exposure using the same saporin concentration. With bleomycin (0.007 IU/ml), cell viability was reduced 3-fold versus drug alone treatment using $AlPcS_{2a}$ (1μg/ml) PCI after 10 min light exposure and $TPPS_{2a}$ (0.1 μg/ml) PCI after 5 min light exposure.

In combination with saporin and bleomycin, the cell viability was significantly reduced after light treatment. The results indicate that PCI has the potential to induce relocalisation of bleomycin and saporin inside cells and thereby enhance cell death.

IL246

Spheroidene in the *Rhodobacter sphaeroides* reaction centre. A surprise

A.C. Wirtz[1], M.C. van Hemert[2], J. Lugtenburg[2], H.A. Frank[3], E.J.J. Groenen[4]

[1]Leiden University, Leiden Institute of Physics, Leiden, The Netherlands; [2]Leiden University, Leiden Institute of Chemistry, Leiden, The Netherlands; [3]University of Connecticut, Department of Chemistry, Storrs, USA; [4]Leiden University, Leiden Institute of Physics, MoNOS, Leiden, The Netherlands

Carotenoids in the membranes of purple photosynthetic bacteria are found both in the light-harvesting complexes and in the photosynthetic reaction centres (RC). Whereas in the light-harvesting complexes the carotenoid is in the all-trans form, when bound to the RC of anaerobically grown *Rhodobacter sphaeroides* spheroidene is long known to adopt a *cis* conformation. Resonance Raman and nuclear magnetic resonance studies of spheroidene reconstituted in the carotenoidless *Rb. Sphaeroides* R26 have indicated that the *cis* linkage concerns the 15,15' carbon-carbon double bond. The crystallization and x-ray diffraction of the RC allowed for ever more accurate determination of the structure of the RC, but the resolution of the electron density maps around the spheroidene molecule does not suffice to unequivocally determine the structure of the carotenoid.

Some time ago we embarked on a project aimed at the determination of the structure of spheroidene in the RC. It involves (1) the synthesis of specific ^{13}C and ^{2}H labeled spheroidenes, (2) the reconstitution of these spheroidenes into the R26 RC, (3) recording resonance Raman spectra of the spheroidene isotopomers both in solution and bound to the RC, and (4) the analysis of the resonance Raman spectra by density-functional theory.

In the talk, I will describe the approach and the analysis of the resonance Raman spectra, which shows that the RC must contain non-planar 15,15'-*cis* spheroidene. We assign the resonance Raman

transition at 1239 cm^{-1}, characteristic for spheroidene in the RC, to a normal mode uniquely coupled to the *cis* nature of the 15,15' carbon-carbon double bond. The detailed analysis of the resonance Raman transitions and their isotope-induced shifts, however, shows that this is not the whole story …

IL247

Molecular basis of photoprotection in higher plants

Bruno Robert

Institute of Biology and Technology of Saclay, Gif/Yvette, France

Through their light-harvesting antenna, plants frequently absorb more solar energy than they can use in photosynthesis. This excess energy has the potential to cause cell damage, such as pigment bleaching and protein inactivation. To minimise photodamage, excitation quenchers rapidly appear in the plant photosynthetic membrane when exposed to high illumination conditions. Until recently, this mechanism was still poorly understood. In the last years, combined use of advanced spectroscopic methods, applied either on isolated light-harvesting complexes or on whole leaves, have yielded a precise picture of the molecular events which underlie photoprotection in higher plants. It was in particular shown that LHCII, the main light harvesting complex of higher plant chloroplasts, has the capability to undergo transformation into a dissipative state by conformational change, and that such transformation does occur *in vivo*, at an extent compatible with the extent of photoprotection observed. In this new structure, a specific carotenoid seems to be responsible of the energy dissipation process. Adaptation of plants to high illumination thus occurs through energy dissipation by a carotenoid molecule.

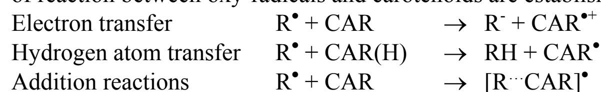
IL248

Are dietary carotenoids anti-or pro-oxidants and are they beneficial or deleterious: a radical story

George Truscott

The Lennard-Jones Laboratories, Keele University, Staffs, UK

Carotenoids such as β-carotene are well known to act with more than one function, e.g. in photosynthesis they are both collectors of light energy and valves to dissipate excess energy. As food supplements there have been many claims and clinical trials that show, or at least suggest, that dietary carotenoids, especially tomato lycopene, lutein and zeaxanthin can help against diseases such as prostate cancer, heart problems and age-related macular degeneration[1,2]. However, there are also well known reports of deleterious effects of high dose supplements of β-carotene on sub populations such as smokers[3]. An explanation for these apparently contradictory effects can be based on the interactions of dietary carotenoids with potentially damaging oxy-radicals. Several types of reaction between oxy-radicals and carotenoids are established:



Electron transfer	$R^{\bullet} + CAR$	$\rightarrow R^{-} + CAR^{\bullet+}$
Hydrogen atom transfer	$R^{\bullet} + CAR(H)$	$\rightarrow RH + CAR^{\bullet}$
Addition reactions	$R^{\bullet} + CAR$	$\rightarrow [R^{\cdots}CAR]^{\bullet}$

These reactions have been studied for the dietary carotenoids via the pulse radiolysis and flash photolysis techniques[4]. One of the most important is electron transfer involving strongly oxidising radicals such as $NO^{\bullet}_2$ (which arise from environmental pollution and cigarette smoke), $NO_2^{\bullet} + Car \rightarrow NO_2^{-} + Car^{\bullet+}$

It is important to note that the carotenoid now contains an odd electron, a carotenoid radical cation, has been formed. The removal of the potentially damaging oxy-radical [e.g. $NO_2^{\bullet}$] is clearly beneficial but we must consider what are the consequences of the production of $Car^{\bullet+}$, this depends on the properties of $Car^{\bullet+}$. Carotenoid Radical anions can also arise ($Car^{\bullet-}$) but these are probably less important. Two important properties of $Car^{\bullet+}$ are lifetime and reactivity and these will be discussed in terms of the potential to do harm i.e. act as a pro-oxidant. The reactivity itself is dependent on the redox potential of the carotenoid radicals and these parameters will be discussed in order to understand the possible molecular mechanisms associated with beneficial and damaging effects of dietary carotenoids. Recent results on radical anions will also be considered[5].

[1]El-Agamey A., Cantrell A., Land, E.J., McGarvey D.J., Truscott T.G., Are dietary carotenoids beneficial? Reactions of carotenoids with oxy-radicals and singlet oxygen. *Photochem. Photobiol. Sci.,* 2004, 3, 802-811. [2]El-Agamey A., Lowe G.M., McGarvey D.J., Mortensen A., Phillip D.M., Truscott T.G., Young J., Carotenoid radical chemistry and antioxidant/pro-oxidant properties. *Arch. Biochem. Biophys.*, 2004, 430, 37-48. [3]The ABC-Study Group, The effect of vitamin E and beta-carotene on the incidence of lung cancers in male smokers. *N. Engl. J. Med.*, 1994, 330, 1029. [4]Bensasson R.V., Land E.J., Truscott T.G., Excited States and Free Radicals in Biology and Medicine. *Oxford University Press* 1993, 1-43l. [5]Edge R., El-Agamey A., Land E.J., Navaratnam S., Truscott T.G., Studies of carotenoid one-electron reduction potentials. *Arch. Biochem. Biophys.*, 2007, 458, 104-110.

T.G.T. thanks Professors E.J. Land and F. Boehm and Drs R. Edge, D. McGarvey, A. El-Agamey, S. Navaratnam and A. Cantrell for their active collaboration.

IL249

From photoprotection to photosensitization - Photoreactivity of the degradation products of carotenoids accumulating in the eye

Malgorzata Barbara Rozanowska[1], Anna Pawlak[2], Bartosz Rozanowski[3]

[1]School of Optometry and Vision Sciences, Cardiff University, Cardiff, United Kingdom; [2]Department of Biophysics, Faculty of Biotechnology, Jagiellonian University, Krakow, Poland; [3]Department of Cell Biology and Genetics, Institute of Biology, Krakow, Poland

Dietary xanthophylls, lutein and zeaxanthin accumulate in human tissues reaching particularly high concentration in the retina where they are believed to play a protective role by filtering out blue light and antioxidant action. However, interaction of xanthophylls with free radicals may lead to xanthophyll degradation. The purpose of our study was to determine whether degradation of xanthophylls under physiologically relevant conditions leads to generation of degradation products of photosensitizing properties.

Lutein or zeaxanthin were incorporated into phosphatidylcholine liposomes and exposed to iron ions and/or visible light. Xanthophyll decay and formation of the degradation products was monitored by HPLC and spectrophotometry. Light-induced generation of free radicals was monitored by nanosecond laser flash photolysis combined with kinetic absorption spectroscopy and by electron paramagnetic resonance (EPR) spin trapping. Time-resolved detection of infrared phosphorescence was used to monitor light-induced formation of singlet oxygen. EPR oximetry was used to monitor the susceptibility of liposomal lipids to photooxidation as a function of irradiation wavelength within a range of 320-600 nm.

Lutein was more susceptible to degradation than zeaxanthin. Upon degradation xanthophylls formed numerous products absorbing at shorter wavelengths than the parent compounds. Degradation products of both lutein and zeaxanthin photogenerated triplet states, free radicals and singlet oxygen. Quantum yields of singlet oxygen photogenerated by excitation with 355 nm or 420 nm laser pulse were up to 29%. Liposomes including degraded xanthophyll were susceptible to blue-light-induced photooxidation and the efficiency of that process was strongly increasing with decreasing irradiation wavelength when normalised to an equal number of incident photons.

In conclusion, while xanthophylls are well known antioxidants, their antioxidant action leads to xanthophyll degradation. Xanthophyll degradation products exhibit opposite properties to their parent compounds – instead of offering antioxidant protection, they act as potent photosensitizers generating singlet oxygen, free

radicals and promoting lipid peroxidation upon exposure to blue and UVA light.

IL250
Oral suncare and photoprotection with carotenoids
Regina Goralczyk
DSM Nutritional Products Ltd., Basel, Switzerland
The normal Western population is increasingly exposing itself to natural and ambient sources of UV-radiation (UVR) due to a lifestyle favouring tanned skin. Chronic exposure to UV radiation leads to epidermal and dermal damage, such as hyperkeratosis, keratinocyte dysplasia and dermal elastosis in affected skin areas, clinically presenting as photoaged skin. To limit the adverse effects of excessive sun exposure, nutritional manipulation of basic skin endogenous protective properties is an attractive proposition. There has been considerable interest in the dietary carotenoids for many years, due to their radical scavenging and singlet oxygen quenching properties and thus their putative role in photochemistry, photobiology and photomedicine.
Carotenoids are natural pigments commonly found in brightly coloured vegetables and fruit. Carrots, apricots and green leafy vegetables such as spinach contain mainly ß-carotene and the carotenoids lutein and zeaxanthin. Tomatoes contain high levels of lycopene.
More recently, carotenoids have received increasing interest as beauty supplements for oral sun care. Carotenoids protect skin from sunlight damage in several ways, including: increasing optical density, quenching singlet oxygen or, for provitamin A carotenoids, formation of retinoic acid, a known topical therapeutic for premature skin aging. Over the past 30 years, a considerable body of evidence has emerged from human, animal and in vitro skin cell studies on the protective effects of carotenoids, demonstrating that they can mildly alleviate sun burn, photo-immune suppression and reduce molecular markers for photoaging. This research is summarized in this review, and new molecular mechanisms, recently identified by nutrigenomics tools, are discussed.

IL251
Influence of molecular interactions on the natural colours of carotenoids
George Britton
University of Liverpool, Liverpool, United Kingdom
As expected of compounds that absorb light maximally in the region 400-450 nm, carotenoids are typically yellow-orange, and provide very familiar examples of yellow and orange colours in Nature. Such examples include yellow flowers e.g. sunflowers, daffodils, marigolds, orange fruit and vegetables such as oranges and carrots, and animals including goldfish, salmon and ladybird beetles. Sometimes, however, the colour seen is not what would be predicted from simple absorption spectra in solution. Tomato fruit are red and flamingo feathers pink, rather than orange. Most striking of all, the purple-blue colours of living crustaceans, corals, starfish and other marine invertebrate animals are also due to carotenoids. How can these unusual and unexpected carotenoid colours be explained? The answer generally lies in molecular interactions that can strongly influence the electronic structure and properties of the carotenoids. In tomato fruit, the red colour seen is attributed to the assembly of the lycopene molecules into microcrystalline assemblies. In other cases the carotenoids are associated with other molecules, especially proteins. Through interactions with keratin, the structural protein of feathers, the same carotenoid may give yellow or red colour in different feathers in the same individual. In the carapace of crustaceans, and in outer tissues of starfish, corals etc, the carotenoid, most commonly astaxanthin, is present in the form of specific carotenoprotein complexes. The most extensively studied of these is crustacyanin, the blue (maximum absorption 630 nm) pigment of lobster shell; cooking the lobster denatures the protein and reveals the orange-red colour of the liberated astaxanthin. Detailed studies by spectroscopic methods and X-ray crystallography have revealed a great deal about the nature of the carotenoid-protein interactions that cause the colour shift, which has been attributed variously to polarization or twisting of the chromophore, or exciton interactions between two carotenoid molecules in close proximity.
Some studies of these various interactions will be described, the evidence evaluated, and some remaining questions highlighted.

IL252
Signaling pathways in sunburn cell formation: from ROS to cell death
An Van Laethem, Kris Nys, Marjan Garmyn, Patrizia Agostinis
Catholic University Leuven, Leuven, Belgium
Chronic exposure to the UVB fraction of sunlight is a major risk factor for skin cancer, one of the most prevalent cancers worldwide. UVB causes mutagenic DNA lesions in the keratinocytes and activates the skin nucleotide excision repair mechanism in the attempt to repair the DNA damage. When the damage is beyond repair or is insufficient, keratinocytes will undergo apoptosis, in a process called sunburn cell formation, as a last escape mechanism. Insights into the molecular mediators which regulate this process and the disturbed pathways underlying the sunburn resistant, carcinogenic phenotype, is a requisite to find effective anticancer therapeutics.
In this study we found that the reactive oxygen species (ROS) generated rapidly after UVB irradiation of human keratinocytes, mediate the swift activation of Apoptosis Signal Regulating Kinase-1 (Ask-1), a redox-sensitive MAP3K, which in turn regulates the p38 MAPK and JNK cascades. The NADPH oxidase antagonist diphenylene iodonium chloride and the EGFR inhibitor AG1487 prevent UVB-mediated ROS generation, the activation of the Ask-1-p38/JNK stress response pathways, and apoptosis, evidencing the link existing between the early plasma membrane-generated ROS and the activation of a lethal cascade initiated by Ask-1. Stable overexpression of Ask-1 sensitizes human keratinocytes to apoptosis following UVB. $p38\alpha^{-/-}$ deficiency or p38 MAPK pharmacological inhibition revert the killing effect of UVB while leaving JNK activation unaltered. These observations indicate that following UVB the pro-apoptotic ROS-Ask-1 cascade is propagated mainly by the activation of the p38 MAPK. Once activated, the Ask-1-p38 MAPK axis is required to engage critical pre-mitochondrial events which lead to the activation of Bax followed by its mitochondrial translocation, cytosolic cytochrome c release and mitochondria generated ROS, which amplify keratinocyte apoptosis. We are currently studying the molecular and functional implications of the p38 MAPK-mediated phosphorylation of p53 at Ser46 as downstream event of this cascade resulting in the regulation of p53 pro-apoptotic function.
Altogether these results argue that ROS act as a crucial second messenger leading to the cytosolic activation of the Ask-1–p38 MAPK pathway, which by culminating in mitochondrial apoptosis, may prevent the propagation of potentially mutagenic keratinocytes following UVB induced damage.

IL253
Detecting Reactive Oxygen Species (ROS) in plants under UV stress
Éva Hideg
Institute of Plant Biology, Biological Research Center, Szeged, Hungary
Ultraviolet (UV) radiation is an increasing environmental challenge, making the responses of all photosynthetic organisms, particularly of agricultural plants to UV-B (280-320 nm) important. Depending on the irradiation dose, the sensitivity of the plant, as

well as on the co-existence of other environmental stress factors, UV radiation may induce oxidative stress. In order to understand the role and possible physiological functions of ROS in UV stress, their direct, in vivo measurement is of special importance. In an increasing order of specificity, the presence of ROS may be verified relying on changes in the blue-green auto-fluorescence of leaves[1], measuring ROS-related ascorbate radical production by EPR[2,3], attempting to apply spin traps in vivo[4], infiltrating the leaf with fluorescent sensors specific to singlet oxygen[5], superoxide[6] or to hydroxyl radicals[7], or trying to detect singlet oxygen infrared photoemission[8].

Publications on UV-related ROS – all co-workers are kindly acknowledged: [1]Hideg É, Juhász M, Bornman JF, Asada K (2002) Photochem. Photobiol. Sci. 1, 934-941. [2]Hideg É, Mano, J, Ohno Ch, Asada K (1997) Plant Cell Physiol. 38, 684-690. [3]Hideg É, Rosenqvist E, Váradi Gy, Bornman J, Vincze É (2006) Funct. Plant Biol. 33, 77-90. [4]Hideg É, Vass I (1996) Plant Sci. 115, 251-260. [5]Hideg É, Barta Cs, Kálai T, Vass I, Hideg K, Asada K (2002) Plant Cell Physiol. 43, 1154-1164. [6]Barta Cs, Kálai T, Hideg K, Vass I, Hideg É (2004) Funct. Plant Biol. 31, 23-28. [7]Šnyrychová I, Kós PB, Hideg É (2007) under review, [8]Hideg É, Melø TB, Naqvi RK, unpublished.

The fluorescent ROS sensors used in this study were synthesized at the Department of Organic and Medicinal Chemistry, University of Pécs, Hungary. For further information contact Dr Tamás Kálai (tamas.kalai@aok.pte.hu) or Prof Kálmán Hideg (kalman.hideg@aok.pte.hu). A special ROS imaging system was developed by Prof Ulrich Schreiber, Universität Würzburg, Germany, (ulrichschreiber@gmx.de) using the standard Imaging-PAM Multi Control Unit IMAG-CM of Heinz Walz GmbH, Effeltrich, Germany (http://www.walz.com). Research was partly supported by the Hungarian National Scientific Research Found (grant No. OTKA T049438).

IL254

Regulation of wound healing and UV response by peroxiredoxin 6

Sabine Werner

ETH Zürich, Zürich, Switzerland

Fibroblast growth factor 7 (FGF7) is a potent cytoprotective growth factor, which is used pharmacologically in cancer patients for the protection of the oral and intestinal epithelium from chemotherapy- and radiotherapy-induced cell damage. We recently showed that FGF7 can also protect keratinocytes of the skin from cell death induced by UV radiation or toxic chemicals in vitro and in vivo. This effect is achieved through regulation of different cytoprotective genes. One of them encodes peroxiredoxin 6, a poorly characterized enzyme, which detoxifies hydrogen peroxide and organic peroxides. Using gain-of-function and loss-of-function approaches we identified important roles of peroxiredoxin 6 in the protection of the epidermis from UV-induced cell death and in cutaneous wound repair.

IL255

UVA-induced reactive oxygen species in human keratinocytes

Irene Kochevar

Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States

Exposure of skin to UVA contributes to development of skin cancer and photoaging and elicits phototoxicity respones. UVA-induced responses are mediated by reactive oxygen species (ROS) in normal skin and in photosensitivity diseases. We investigated the source(s) of these ROS in normal human keratinocytes (HK) and in keratinocytes that mimic cells in the UVA photosensitivity associated with Smith-Lemli-Opitz (SLO) syndrome. We tested the hypothesis that NADPH oxidase is a major source of UVA-induced ROS. After treatment of HK with 5 J/cm^2. UVA, the ROS level and the NADPH oxidase activity increased with similar kinetics (maximum at 15 min) and the ROS was partially blocked by DPI, a NADPH oxidase inhibitor, and to a much lesser extent by MitoQ, a mitochondria-selective antioxidant. Depleting the Nox1 isoform of the catalytic subunit of NADPH oxidase using siRNA blocked the UVA-induced ROS increase and PGE2 synthesis. Activation of NADPH oxidase is mediated by an increase in intracellular calcium and ceramide, which has been proposed to mediate responses to UVA in HK, also activated NADPH oxidase.

High levels of 7-dehydrocholesterol (7-DHC), the precursor to cholesterol, cause exaggerated photosensitivity to UVA in patients with SLO syndrome. To produce cells mimicking SLO keratinocytes, HK were partially depleted of cholesterol and supplemented with 7-DHC. UVA treatment of SLO-HK elicited much a greater level of ROS, NADPH oxidase activity, intracellular calcium, PLA2 activity and PGE2 than treatment of normal HK. UVA-induced ROS and PGE2 production were inhibited in these cells by depleting the Nox1 subunit of NADPH oxidase using siRNA. Partial replacement of cholesterol with 7-DHC also disrupted membrane lipid raft domains, although depletion of cholesterol, which also disrupts lipid rafts, did not affect UVA-induced increases in ROS and PGE2. These results indicate that Nox1 is the major source of UVA-induced ROS in both normal and photosensitive keratinocytes.

OC256

Melanocortin 1 receptor (MC1R) genotype influences erythemal sensitivity to PUVA

Gillian Smith[1], Murray JV Wilkie[1], Yusuf Y Deeni[1], Peter M Farr[2], James Ferguson[3], C Roland Wolf[1], Sally H Ibbotson[3]

[1]Biomedical Research Centre, University of Dundee, Ninewells Hospital & Medical School, Dundee, Scotland, UK; [2]Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne, UK; [3]Photobiology Unit, Ninewells Hospital & Medical School, Dundee, Scotland, UK

The melanocortin 1 receptor (MC1R) is a highly polymorphic G-protein-coupled receptor. Inheritance of various MC1R alleles has been associated with a red hair/fair skin phenotype, increased incidence of skin cancer and altered sensitivity to ultraviolet radiation (UVR). We have investigated whether MC1R genotype also influences PUVA erythemal sensitivity in patients with psoriasis and a variety of other common skin diseases. Patients (n=111) about to start PUVA photochemotherapy were recruited to our study, and erythemal responses assessed visually at 72 h and 96 h following PUVA by assessment of the minimal phototoxic dose (MPD). MC1R genotype was determined by direct sequencing. Inheritance of the MC1R $Arg_{151}Cys$ SNP was associated with a red hair phenotype (Odds Ratio (OR) 25.19, p=0.0004). In contrast, inheritance of the $Val_{60}Leu$ and $Arg_{163}Gln$ SNPs was associated with increased PUVA erythemal sensitivity (reduced MPD) 72 h following treatment (n=111; $Val_{60}Leu$ χ^2=5.764, p=0.016, $Arg_{163}Gln$ χ^2=5.469, p=0.019 and in a sub-set of patients with psoriasis, n=55; $Val_{60}Leu$ χ^2=4.534, p=0.033, $Arg_{163}Gln$ χ^2=7.298, p=0.007). Inheritance of two or more MC1R SNPs was also associated with increased PUVA erythemal sensitivity (reduced MPD) (n=111; χ^2=8.166, p=0.017 and in a sub-set of patients with psoriasis, n=55; χ^2=10.303, p=0.016). In addition, quantitative RT-PCR analysis demonstrated that MC1R mRNA expression significantly correlated with additional PUVA-induced genes, including glutathione S-transferase P1 (GSTP1) and cytochrome P450 CYP2S1. Our data suggest that MC1R genotype influences erythemal sensitivity following PUVA exposure and suggest that MC1R may also be regulated as part of a co-ordinated cellular adaptive response to oxidative challenge.

IL257

Diurnal and circadian expression of genes encoding opsin-like photoreceptor proteins in *Hydra* (Cnidaria, Hydrozoa)

Silvia Santillo[1], Pierangelo Orlando[2], Luciano De Petrocellis[1], Carlo Musio[1]
[1]Istituto di Cibernetica, Pozzuoli, NA, Italy; [2]Istituto di Biochimica delle Proteine del CNR, Napoli, Italy

Visual pigment research provides evidence that animal photopigments are ubiquitously GPCR (G protein-coupled receptors) opsin-like proteins, which are functionally included into seven subfamilies: 1. vertebrate visual and non-visual opsins; 2. encephalopsins; 3. G_q opsins/melanopsins; 4. G_o opsins; 5. neuropsins; 6. peropsins; 7. retinal photoisomerases. Surprisingly, the opsins' highest percentage belongs to non-image-forming visual systems triggering the non-visual photoreception, once termed extraocular photoreception. Non-visual photoreceptor systems detect environmental light irradiance, interact with the image-forming pathways and regulate time-dependent physiological processes modulated by diurnal and circadian control of the light input. Since our first immunochemistry finding of a rhodopsin-like protein in the cnidarian Hydra - an eyeless old metazoan showing only a non-visual photoreception and the first appearance of a nervous system - we were aimed to search for putative ancestral issues of both visual and non-visual photoreceptive systems and to demonstrate that circadian and diurnal regulation of the photic input occurred at early stage of animal evolution. Sequences of vertebrate-like short-wavelength opsins (GenBank CN770545 and CN554795), peropsin (GenBank CB073527) and melanopsin (GenBank DT617488) were selected among those available in the Hydra EST database. Quantitative real-time PCR was used to detect their mRNA amounts in animals sacrificed at 3h intervals of both 12:12h LD (alternating light/dark) cycle for diurnal rhythm (occurring during day-time light) and 12:12h DD (constant darkness) for circadian rhythm (persisting with a 24h cycle under constant light conditions) experiments. In both diurnal and circadian cycles, oscillatory patterns of gene expression of all examined photopigments were observed. All opsin expression levels were regularly periodic during the tested cycles approaching to a minimum peak around sunset for diurnal cycle and at CT (circadian time) 15 and 24 for circadian one. Diurnal fluctuations reflected the contraction-burst activity variation of the animal's behavioural cycle. Our results hint the existence of circadian clock elements in Hydra and are consistent with the rhythmic regulation of known animal visual and non-visual opsins suggesting that the photoregulation of opsin expression occurs in lowest Metazoa and results conserved during phylogenesis. We are confident that Hydra opsin(s) could be a skeleton key suitable for evo/devo study of photoreceptive systems and correlated behaviours.

IL258

Confocal microscopy in ciliate protozoa

Giuliano Colombetti[1], Paolo Bianchini[2], Ranieri Bizzarri[3], Giovanni Checcucci[1], Alberto Diaspro[2], Sabina Lucia[1], Mattia Pesce[2], Paola Ramoino[4], Cesare Usai[1], Giuseppe Vicidomini[2]
[1]CNR Istituto di Biofisica, Pisa, Italy; [2]LAMBS-MicroScoBio, Dip. Fisica, Univ. Genova, Genova, Italy; [3]Scuola Normale Superiore, Pisa, Italy; [4]DIPTERIS, Università di Genova, Genova, Italy

Many ciliates are able to respond to light stimuli by altering their motion parameters (photomovement). Among them, the heterotrichs *Blepharisma japonicum*, *Fabrea salina* and *Stentor coeruleus* are colored (red, black and cyan, respectively), their color being due to pigments belonging to the hypericin family. It is currently thought that these pigments are the photoreceptor pigments responsible of the photomovement. It has been suggested that a differential distribution of the pigment along the cell body might explain the photoresponse. Since the pigments are highly fluorescent, we have investigated their fluorescent properties as a function of the cell body localization by means of single and two-photon confocal microscopy. Besides the species mentioned above, we have also investigated a laboratory generated form of *B. japonicum*, called blue for the color it assumes after exposure to visible light in the presence of oxygen. Our results have shown, to our surprise, that besides the expected presence in the cortical region immediately below the cell membrane, the pigments are also distributed along the cilia. To the best of our knowledge, this is the first time that this evidence is described in literature. Potential implications for the process of photosensory transduction are briefly discussed. In order to ascertain possible differences in the pigments fluorescence properties along the cell body, we have measured emission and excitation spectra from different parts of it (anterior, posterior and cilia). Our results clearly indicate that in all cases the spectra are the same, within experimental errors. After this, we have determined by means of fluorescence lifetime imaging (FLIM) the distribution of fluorescence lifetimes along the cell body. The results show that in all cases considered there are two fluorescence lifetimes, one of the order of some hundred ps and the other between 1.2 ns and 2.3 ns, depending on the cell type, homogeneously distributed along the cell body with the exception of *F. salina*, in which case there is a longer lifetime (of the order of 2.3 ns) in the adoral membranelles with respect to the cell body (1.8 ns). We have then evaluated the pigment relative fluorescence efficiency of these ciliates. In an ordered scale from lower to greater efficiency, we have *S. coeruleus*, *B. japonicum* blue, *B. japonicum* red and *F. salina*. Preliminary fluorescence anisotropy measurements show a clear anisotropy mostly localized on the cell membrane, with value of about 0.11.

PL301

From photochemistry and photobiology to photobiotechnology of plants

Pill-Soon Song[1,2]
[1]Cheju National University, Jeju, Jeju-Do, Korea; [2]EBRC/Gyeongsang National University, Jinju, Kyungnam, Korea

The search for sunlight is critical for plant growth and development. Plants grown under shade/far-red (FR) light stresses such as under a forest canopy or in close proximity to neighbor plants experience a decrease in the R:FR ratio. Reduction of this ratio induces the plants to avoid the shade in search of photosynthetically actinic R light. The shade-avoidance reactions include a rapid increase in the extension growth of stems and petioles at the expense of leaf growth, storage organ production, and reproductive development. Phytochromes, the R:FR-sensing photoreceptor, regulate the shade-avoidance reaction in response to the R:FR ratio or photoequilibrium between the R-absorbing Pr and the FR-absorbing Pfr forms. In FR light environments the photoequilibrium of phytochrome is shifted toward the Pr form that induces shade avoidance responses. From an understanding of the structure and function relation of the phytochromes, we developed mutant phytochromes that enable the plants to suppress the shade-avoidance responses and confer the shade-tolerance to the plants. Genetically engineered plants containing such a mutant phytochrome can be used for the development of crop plants with enhanced yields and other useful traits. We developed both hyperactive phytochrome A (S598A mutant phyA) and bathochromic mutant phyA. The latter's Pr-absorbance band maximum was shifted to a longer wavelength by a few nanometers. The bathochromic shifts allow activation of phyA even at low R:FR ratios to suppress plants' shade avoidance phenotypes. The *in vivo* function of the phyA mutants and their shade resistance role have been demonstrated by using transgenic *Arabidopsis thaliana* plants. The shade-tolerance trait offers attractive biotechnological potentials in various shade-sensitive plants such as turf grass and crop plants.

(Supported by BioGreen 21/Rural Development Agency and NCRC/GSNU).

IL302

A novel role for c-Fos in the defence against ultraviolet light

Bernd Kaina

University of Mainz, Department of Toxicology, Mainz, Germany

Cells deficient in c-Fos are hypersensitive to the cytotoxic and genotoxic effects of ultraviolet (UV-C) light. Here we demonstrate that mouse embryonal fibroblasts lacking c-Fos (*fos-/-*) are defective in the repair of UV-C induced DNA lesions: they show a decreased rate of sealing of DNA strand breaks that arose during nucleotide excision repair (NER) and are unable to remove cyclobutane pyrimidine dimers (CPDs) from DNA. A search for genes responsible for the hypersensitivity and the DNA repair defect of c-Fos deficient cells by means of DNA repair micoarray revealed a difference in the expression of the endonucleases XPF and XPG upon UV-C exposure. UV-C reduced the expression of *xpf* and *xpg* mRNA in both cell lines, however in the wild-type (wt) re-synthesis occurred promptly whereas in the mutant resynthesis was impaired. In consequence, the XPF protein was not detectable 6-18 h after irradiation in c-Fos deficient cells whereas it recovered quickly in the wt. In *fos-/-* cells the impaired removal of CPDs was shown to induce apoptosis via sustained activation of JNK and long lasting induction of the *fasL*. Inhibition of early JNK activation by the JNK inhibitor SP600125 increased UV-C induced apoptosis whereas inhibition of late JNK activation in *fos-/-* cells attenuated the apoptotic response. Overall, the data show that the immediate-early induction of the JNK/*c-fos* pathway stimulates CPD removal by resynthesis of XPF and XPG mRNA and protein. Thus, c-Fos/AP-1 does not provoke genuine induction of the xpf and xpg gene but rather stimulates their transcriptional activity in response to genotoxic stress (gene homeostasis). If this fails, repair of UV-C induced DNA lesions does not occur causing sustained transcriptional inhibition that leads to a reduced expression of MPK1, long-lasting JNK activation, *fasL* overexpression and finally apoptosis by activation of the death receptor pathway.

Work supported by DFG-Ka724 and SFB432.

IL303

Molecular responses of normal human keratinocytes in culture and in reconstructed skin to genotoxicity induced by solar UV: towards a genomic protection factor *in vitro*

Laurent Marrot, Jean-Philippe Belaidi, Christophe Jones, Jean-Roch Meunier

Phototoxicity Unit, Safety Research Dpt, L'OREAL, Aulnay-sous-Bois, FRANCE

Keratinocytes constitute one of the most critical targets for solar radiation and this cell type is implicated in development of photo-induced skin cancers such as carcinoma. Many in vitro studies have been reported about molecular mechanisms involved in UV-induced keratinocyte transformation. In this context, we have compared the impact of UV on human normal keratinocytes of the same origin either in culture or in reconstructed skin. In realistic exposure conditions in terms of spectral power distribution (UVB + UVA from a solar simulator) and at doses where cell survival was over 50% as compared to unexposed control, we observed that (i) UV-induced DNA breakage assessed by comet assay immediately after irradiation was higher in reconstructed skin than in culture, (ii) 5 hours post-exposure, accumulation and phosphorylation of p53 were comparable in both models. However, 24 hours later, p53 remained detectable only in keratinocytes in culture but no longer in reconstructed skin, (iii) among the genes controlled by p53 we have studied, GADD45 was the only one highly expressed in reconstructed skin whereas P21 and MDM2 were predominant in cultured cells. These data suggest that molecular responses to UV in keratinocytes can be influenced by their biological environment. Finally, in both systems, the genomic protection (DNA damage and p53 status) provided by three marketed formulations claiming a sun protection factor (SPF) value of 15 was studied. It was shown that, beyond SPF, photostability and UVA screening efficiency were essential for an efficient protection against photogenotoxicity.

IL304

Viral vectors to explore the consequences of UV-induced DNA damage

Carlos FM Menck

Dept. of Microbiology, Institute of Microbiology, University of Sao Paulo, Sao Paulo, SP, Brazil

The ultraviolet component of sunlight (UVB) generates two major lesions in the DNA molecule: cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine-pyrimidone (6-4PPs). Both types of DNA lesion disturb fundamental cellular processes, such as DNA replication and RNA transcription, and one longstanding question is their relative roles as primary agents of skin injuries by sunlight, such as aging and carcinogenesis. Two recombinant adenovirus vectors carrying photolyases genes were constructed and used to answer this question, as photolyases are specific for the photorepair of CPD (from the rat kangaroo Potorous tridactylus) or 6-4PP (from Arabidopsis thaliana) lesions. Both photolyases were efficiently transduced in human fibroblasts and able to prevent UV-induced apoptosis in NER-deficient cells to a similar extent; and when these photolyases were co-expressed in NER-deficient cell lines, photorepair decreased apoptosis to levels similar to unirradiated cells. However, in NER- proficient cells, including XP-V cells, UV induced-apoptosis was prevented only after photorepair in cells expressing CPD-photolyase, with no effects observed in the photorepair of 6-4PP by the specific photolyase. Taken together, these results strongly suggest that while both CPDs and 6-4PPs contribute to UV-induced apoptosis in NER-deficient cells, CPDs seem to play the major role triggering the UV-killing responses in NER-proficient human fibroblasts, probably due to the fast repair of 6-4PPs. As a consequence, the difference in skin photosensitivity, including carcinogenesis, of most of the xeroderma pigmentosum patients (NER-deficient) and of normal people (NER-proficient) is probably not a quantitative aspect, but depends on the type of DNA damage induced by sunlight.

Financial support: FAPESP (São Paulo, Brazil), CNPq (Brasília, Brazil), John Simon Guggenheim Memorial Foundation (New York, USA).

IL305

Molecular mechanisms of UV-induced mutagenesis in human cells

Alain Sarasin[1], Anne Stary[1], Quentin Gueranger[2], Claude-Agnès Reynaud[2], Jean-Claude Weill[2]

[1]Institute Gustave Roussy and FRE2939 CNRS, Villejuif, France; [2]INSERM U783 and Faculté Necker, Paris, France

Several patients exhibit UV-sensitivity caused by some type of DNA repair deficiency. Classical xeroderma pigmentosum (XP) patients do not excise UV-induced DNA lesions. Mutations observed in UV-irradiated XP cells are, therefore, a direct image of error-prone translesion synthesis (TLS) across all types of unrepaired UV-induced damage. XP variant patients (XPV) are deficient in the TLS polymerase eta able to replicate opposite TT dimers with few mistakes if any. Mutations observed in XPV cells reflect the TLS activity of polymerases other than DNA polymerase eta. All these patients develop numerous UV-induced skin cancers. Mutation spectra observed in some key genes isolated from these tumours, such as the p53 tumour suppressor gene, reflect the in vivo consequences of TLS across the lesions. Analysis of these tumours confirms that UV-induced mutagenesis is basically characterized by C to T transitions localized at dipyrimidine sites. CC to TT tandem mutations represent the signature of UV-induced mutagenesis. Their frequencies are exacerbated in classical XP cells or tumours, probably due to an increase of cytosine deamination with time, leading to uracile that is replicated by the polymerase eta as a T. In XPV cells or tumours, increased mutagenesis is observed, as compared to normal cells, because the relatively error-free polymerase eta is absent and replaced by another TLS polymerase more error-prone, especially on T-containing dimers. The level of C to T transitions decreases as compared to classical XP as well as

the tandem frequency. To analyze the role of other TLS DNA polymerases in UV-induced mutagenesis, Burkitt's lymphoma BL2 cells have been engineered to specifically knock-out, by homologous recombination, the polymerases eta, iota and zeta. Analysis of the mutation spectra, using UV-irradiated shuttle vectors replicating in these three types of cells, reveals the role and properties of these three TLS polymerases in vivo during the bypass of UV-induced DNA lesions. All the data have been confirmed by re-expressing the wild-type TLS polymerase gene in the corresponding KO cells, showing the recovery of normal phenotype, including frequency and spectra of UV-induced mutagenesis. These results allow us to better understand the molecular mechanism of UV-induced mutagenesis and data concerning the properties of several TLS DNA polymerases may shed light also on the origin of other types of cancers.

OC306

The UV-B damage response in *Daphnia* spp.: diverse repair strategies at 10 and 20 °C

Sandra J Connelly[1], *Craig E Williamson*[2], *David L Mitchell*[3]
[1]Miami University of Ohio, Department of Zoology, Oxford, OH, USA; [2]Miami University, Oxford, OH, United States; [3]The University of Texas MD Anderson Cancer Center, Smithville, TX, USA

Single-dose, acute UV experiments permit the differentiation of photoprotective and recovery mechanisms in organisms that are otherwise concurrent in nature. This strategy was used to investigate UVB-induced DNA damage induction and repair (photoenzymatic repair, PER, and nucleotide excision repair, NER) rates in four *Daphnia* spp. at two temperatures (10 and 20°C) by quantifying cyclobutane pyrimidine dimers (CPDs) using a radioimmunoassay (RIA). Induction of CPDs following exposure to 1, 2, 3, and 4 kJ m^{-2} acute UVB and repair of damage induced by 2 kJ m-2 acute UVB over a 24 hr period were quantified for *D. pulicaria*, *D. pulex*, *D. middendorffiana* and *D. parvula* (two clones). All species showed high levels of photoprotection (as compared to associated purified DNA dosimeters). In nearly all species examined, PER was more efficient than NER, with NER most variable under both temperature regimes. However, PER showed low temperature dependence in all species, whereas NER was temperature dependent only in some species. Rates of NER and PER were comparable in *D. pulicaria* and *D. pulex* at 10°C, but the rate of NER was much greater than PER in *D. middendorffiana* and *D. parvula*. These data suggest that NER may be more important than PER in the response of *Daphnia* to UV induced DNA damage under colder conditions, such as below the thermocline or in high altitude freshwater systems.

OC307

Association of the extracellular chaperone clusterin with altered elastic fibers *in vivo* and *in vitro*

Elke Janig[1], *Martin Halsbeck*[2], *Johannes Buchner*[2], *Ariane Aigelsreiter*[3], *Helmut Denk*[3], *Kurt Zatloukal*[3], *Peter Wolf*[1]
[1]Institute of Dermatology and Venerology, Medical University of Graz, Auenbruggerplatz 8, 8036 Graz, Austria; [2]Institute of Chemistry, Technical University of Munich, Garching - Munich, Germany; [3]Institute of Pathology, Medical University Graz, Graz, Austria

Clusterin is a secreted chaperone with stress-induced expression in various diseased and aged tissues. Since dermal connective tissue alterations occur due to ageing and ultraviolet (UV) radiation and since we found clusterin in association with elastic material in cirrhotic human livers, we aimed to obtain closer insights into the interaction of clusterin with elastin in intrinsically and extrinsically aged skin. Therefore, we performed histological and immunohistochemical studies of young and aged as well as sun-exposed and -protected human skin. All cases of sun-aged skin showed a massive accumulation of elastotic material (solar elastosis) with strong immunohistochemical staining and constant co-localization of clusterin. In intrinsically aged skin clusterin was associated with thickened elastic fibers. The immunoreaction of clusterin correlated with the extent of abnormal elastin. In contrast, young sun-exposed and -protected skin had a well-organized elastin fiber network, which was constantly negative for clusterin. Because of the striking co-localization of clusterin with abnormal elastotic material, we investigated the interaction of clusterin and elastin *in vitro*. To explore a possible chaperone action of clusterin, we performed an *in vitro* assay, where elastin was treated with UV light in the absence or presence of clusterin. Whereas UV irradiation of elastin resulted in degeneration and aggregation of elastin, addition of clusterin effectively inhibited the formation of elastin aggregates. The interaction of both proteins was further analyzed by size exclusion chromatography, mass spectrometry and electron microscopy, where clusterin was found in a stable complex with elastin after UV exposure. In summary, we show that clusterin specifically co-localizes with altered elastic fibers *in vivo*, especially in chronic UV-damaged skin. In addition, clusterin prevented UV-induced aggregation of elastin *in vitro,* providing evidence that elastin is a target protein of clusterin.

OC308

After UVB irradiation, p21 expression is regulated by p53 through DNA-protein contact and protein-protein interactions

Nathalie Bastien[1], *Walid Dridi*[1], *Isabelle Paradis*[1], *Mylene Blais*[2], *Raouf Fetni*[3], *Claude Asselin*[2], Regen Drouin[1]
[1]Universite de Sherbrooke, Faculty of Medicine and Health Sciences, Service of Genetics, Department of Pediatrics, 3001, 12th Avenue North, Sherbrooke, Quebec, Canada; [2]Universite de Sherbrooke, Faculty of Medicine and Health Sciences, Department of Anatomy and Cellular Biology, 3001, 12th Avenue North, Sherbrooke, Quebec, Canada; [3]Department of Pathology, Hospital Sainte-Justine, Montreal, Quebec, Canada

The p53 protein regulates the expression of many genes implicated in different cell functions including cell cycle checkpoint, DNA repair, differentiation, angiogenesis and apoptosis. This regulation can occur via two different pathways. The first is mediated through direct DNA binding of p53 to its response element (RE) and the second is through a protein-p53 interaction, the protein being bound to DNA. Many *in vitro* analyses have shown p53 binding to its RE. However, the same DNA-protein contact at the RE has not been clearly demonstrated in living cells. The mechanism of p53 regulation is still not fully understood. This led us think that indirect binding of p53 may play an important role in the regulation of its downstream effectors. To understand the mechanisms of p53 regulation, we studied one of its important effectors, *p21*. Human fibroblasts were exposed to 1000 J/m^2 UVB to induce p53. Then, at different times cells were treated with DNase1, DNA was purified, and ligation-mediated PCR (LMPCR) was carried out of the *p21* promoter. Also, 8 h after p53 induction, the Chromatin Immunoprecipitation (ChIP) assay was performed using p53 or Sp1 antibodies, and PCR of different parts of the *p21* promoter was performed . The LMPCR showed that there is no evidence of direct p53 binding to its RE on *p21* in normal human fibroblasts. However, the ChIP showed that: 1) p53 is immunoprecipitated together with its RE on *p21* and 2) p53 is immunoprecipitated together with the Sp1 RE on *p21*. These results suggest that p53 can regulate *p21*through DNA binding (pathway 1) and probably by interaction with Sp1 (pathway 2). To further our understanding of this regulating mechanism, we plan to study the *p21* regulation by p53 using different types of cells following different induction methods, such as ionizing radiation, tubulin inhibitors and 5-fluorouracil.

IL309

The photosensitized production and optical detection of singlet oxygen with sub-cellular resolution in single cells

Peter R. Ogilby

Aarhus University, Department of Chemistry, Aarhus, Denmark

Singlet oxygen, the lowest excited electronic state of molecular oxygen, is an important intermediate in many chemical processes. Singlet oxygen is conveniently produced in a photosensitized process wherein energy is transferred from a photo-excited molecule (the sensitizer) to ground state oxygen. In complex biological systems, the behavior of singlet oxygen thus produced can be influenced by microscopic heterogeneities and phase-separated domains. Thus, much can be gained if, in such systems, singlet oxygen can be both selectively created and then directly monitored in time- and spatially-resolved experiments.

We have embarked on a multi-faceted program in which a variety of techniques are used to examine the behavior of singlet oxygen in micro-heterogeneous systems. In this lecture, I will briefly describe our latest work on the following topics: (a) The creation and detection of singlet oxygen at the sub-cellular level in single cells, (b) optical, electrochemical, and biochemical methods to selectively control the production and deactivation of singlet oxygen with both spatial and temporal resolution, and (c) oxygen diffusion in cells.

Among other things, our results provide a new perspective for mechanistic studies of photoinduced intracellular signaling and cell death which, in turn, have practical applications in Photodynamic Therapy.

IL310

Singlet oxygen production by proteins from the green fluorescent protein family

Santi Nonell[1], Ana Jiménez-Banzo[1], Johan Hofkens[2], Cristina Flors[2]

[1]Universitat Ramon Llull, Institut Quimic de Sarria, Barcelona, Spain; [2]Department of Chemistry and Institute for Nanoscale Physics and Chemistry (INPAC), Katholieke Universiteit Leuven, Leuven, Belgium

Proteins from the family of the green fluorescent protein (GFP) have become popular as genetically encoded reporters for intracellular dynamics, protein expression, protein-protein interaction studies based on fluorescence microscopy and for genetically-targeted chromophore-assisted light inactivation. However, extended observation of GFPs is limited by photobleaching/photoconversion of the chromophore or light-induced damage of the surrounding biological medium. Photoproduction of reactive oxygen species (ROS) can play a role in this limitation. We report an investigation of singlet oxygen photoproduction by GFPs using time-resolved detection of the NIR phosphorescence of singlet oxygen at 1275 nm. We have chosen EGFP as model protein for this study, and we have compared the results with those on the model compound of the EGFP fluorophore 4-hydroxybenzylidene-1,2-dimethylimidazoline (HBDI). We have detected singlet oxygen generated by enhanced (E)GFP, and measured a lifetime of 4 μs in deuterated solution. By comparison with the model compound of the EGFP fluorophore 4-hydroxybenzylidene-1,2-dimethylimidazoline (HBDI), our results confirm that the β-can of EGFP provides shielding of the fluorophore and reduces the production of this reactive oxygen species. In addition, our results yield new information about the triplet state of these proteins.

IL311

Depolarization of cross-membrane electric potential in photosensitization

Benjamin Ehrenberg, Shoshana Bernstein

Bar Ilan University, Department of Physics, Ramat Gan, Israel

In the process of damage to cells during PDT, depolarization of membrane potential is observed. Depolarization can result from photosensitized oxidation of vital proteins located in the cell membrane or inside the cell. It could also result from photooxidation of phospholipids that assemble the membrane, leading to changes in the intactness of the membrane and to ion leakage trough it. This naturally abolishes the electric potential of the membrane by dissipating cross-membrane ion gradients. In this work we examined the extent of membrane damage caused by the photosensitization of liposomes of different phospholipids compositions. The phospholipids varied in the length of the alkyl-carboxylic acid chains and in the number and location of double bonds in the chains. Cross-membrane Nernst potential was induced by K^+-diffusion by the ionophore valinomycin and it was monitored with the fluorescent dye DiS-C_2-(5), a potentiometric indicator. The fluorescence intensity of the dye decreases drastically upon setting the potential and it then increases very slowly due to leakage of ions. The kinetics of this leakage were correlated with the duration of photosensitization by hematoporphyrin, which was used as a sensitizer, for each lipid composition. We found a correlation between the lipid type and the extent of potential decrease following photosensitization. When liposomes were assembled from saturated lipids, such as DMPC, or from natural lecithin mixed with DMPC, photosensitized leakage of liposomes was minimal. This was also the case with lipids that have a single double bond (14:1, 16:1 and 18:1 lipids). A distinct depolarization was observed only when poly-unsaturated lipids, such as having 18:3 and 20:4 structures, were used. DABCO, a specific quencher of singlet oxygen, was employed to verify that singlet oxygen was the main cause for damaging the membrane and for the potential leak, indicating to a Type II mechanism. We conclude that when the lipid composition is similar to that found in natural cell membranes, the electric field that is sustained on liposomes is not amenable to depolarization by singlet oxygen photosensitization. The electric field can be depolarized only when poly-unsaturated lipids, at concentrations that are not usual in natural membranes, are employed.

IL312

Analysis of ALA-induced PPIX across multiple cell lines: variance and dependence upon physical parameters

Summer L. Gibbs, Scott C. Davis, Julie A. O'Hara, Brian W. Pogue

Thayer School of Engineering, Dartmouth College, Hanover, New Hampshire, USA

Inter cellular variation in Protoporphyrin IX production was examined in 10 tumor cell lines in vitro, looking for predictors of production yield and heterogeneity. Then a select group of glioma tumors were grown in nude mice to study the production yields, using fluorescence imaging ex vivo and MRI-guided fluorescence tomography. It was shown, that ALA-PpIX production was most highly correlated to cell size in vitro, and only weakly to other parmeters such as number of mitochondria. Addition of an iron chelator only increased PPIX generation in those cells which had lower endogenous production yields a priori. In vivo, the fluorescence to transmission ratio signal is shown to be predictive of the tumor presence and size, and this ratio data set was used to quantify fluorescence in vivo, and reconstruct images of the yield from non-invasive tomographic measurements. The in vivo imaging illustrated that certain tumor lines produce PPIX predominantly in the normal brain, outside the tumor margin, whereas others produce high fractions in the tumor as well. The heterogeneity of production between lines and even within a single

line requires better analysis, to further aid in the understanding of how fluorescence guided resection may be applied in neurosurgery.

OC313

Time resolved luminescence and singlet oxygen formation under illumination of hypericin in complex with low density lipoproteins

Peter Gbur[1], Daniel Jancura[1], Roman Dedic[2], Dusan Chorvat jr.[3], Jan Hala[2], Pavol Miskovsky[3]

[1]Department of Biophysics, Safarik University, Kosice, Slovakia; [2]Department of Chemical Physics and Optics, Charles University, Prague, Czech republic; [3]International Laser Center, Bratislava, Slovakia

Photosensitized generation of singlet oxygen ($^1O_2$) by molecules of a photosensitizer (pts) is a crucial process in photodynamic therapy (PDT). Upon administration into blood stream photosensitizers associate predominantly with serum proteins. Hypericin (Hyp), a natural photosensitizing agent occurring in the plants of the genus Hypericum, associates mainly with low density lipoproteins (LDL) and to a lesser extent with human serum albumin. In this study we present time–resolved fluorescence and phosphorescence study of Hyp in complex with LDL as well as the evolution of $^1O_2$ formation under illumination of Hyp/LDL complex. Special apparatus for infrared phosphorescence measurements were engaged to monitor Hyp phosphorescence (895 nm) and $^1O_2$ formation and decay (1278 nm) under illumination of Hyp in complex with LDL at different Hyp/LDL molar ratios. It was demonstrated that amount of produced $^1O_2$ as function of Hyp/LDL concentration ratio shows saturation behavior, therefore we assume that the amount of produced $^1O_2$ depends on the quantity of monomeric molecules of Hyp inside the lipidic part of LDL, which performs similar characteristics. The dependence of the intensity of Hyp phosphorescence on Hyp/LDL ratio is alike to that for $^1O_2$ formation. Phosphorescence decay of Hyp in the presence of LDL manifests a non-trivial kinetics. We suppose that the processes of triplet-triplet annihilation and the production of superoxide anion radical ($O_2^{\cdot-}$) contribute to the depopulation of Hyp triplet state. However, the decay process can be satisfactory fitted by a bi-exponential curve and the shorter lifetime component of Hyp phosphorescence matches very well with the rise time of $^1O_2$ generation. Observed shortening of the fluorescence lifetime of Hyp above Hyp/LDL molar ratio 25:1 suggests quenching of excited singlet state of monomeric Hyp at higher Hyp/LDL concentration ratios. This result is consistent with our previous observation of the quenching of the intensity of Hyp fluorescence at high Hyp/ LDL ratios {> 30:1) (Kascakova et.al. Photochem.Photobiol. 81, 1395-1403, 2006).

This work was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic under the grant VEGA No. 2278, by the Slovak Research and Development Agency under the contract APVT-20-036104 and by the project MSM0021620835 from the ministry of Education of the Czech Republic.

OC314

Role of plasma LDL and tumoral microenvironment in tetrapyrrole-photosensitizers cellular uptake: a physico-chemical approach

Stéphanie Bonneau, Halina Mojzisova, Christine Vever-Bizet, Daniel Brault

Université Pierre et Marie Curie - Paris 6, Paris cedex 05, France

Thanks to their preferential retention in proliferating tissues, some photosensitizers are therapeutically used such as in photodynamic therapy (PDT). In most cases, they are based on the porphyrin structure, but other compounds, of which far-red-light absorption properties are most compatibles with biological tissues irradiation, have been developed. In this work, the focus is given on three amphiphilic tetrapyrroles: deuteroporphyrin (DP) that bears two carboxylic groups on one side of the macrocycle, clorin e6 (Ce6), that bears three carboxylic groups on one side, and disulfonated aluminum-phthalocyanine (AlPcS2a) that bears two sulfonated groups on one side. Indeed, the selectivity of photosenzitizers for cells in proliferation, as well as their intracellular localization, depends on their structure, in particular on their hydrophobicity and the symmetry of distribution of their polar chains around the macrocycle. Two major processes are involved. On one hand, lipophilic or amphiphilic photosensitizers possess a high affinity for low-density lipoproteins (LDL). The increased cholesterol catabolism of proliferating tissues results in over-expression of LDL receptors. Hence, LDL could act as natural carriers of photosensitizers and insure their targeting to tumor cells. On the other hand, the relative acid pH of extracellular medium could play an important role by governing the physico-chemical properties of photosensitizers and consequently their ability to cross membranes. In fine, the photosensitizers behavior seems to involve both the physicochemical and biological properties of the microenvironment.

In order to elucidate the implied mechanisms, we studied the interactions of photosensitizers with LDL and with liposomes (SUV, used as membranes-models) with a special interest on dynamics of these processes. A particular attention has been paid to the effects of pH. The data obtained on these simple systems then allowed us to interpret the sub-cellular localization of the photosensitizers on a human line of fibroblasts, and to evaluate the influence of LDL on the intracellular distribution of the compounds. This last point is of major importance because the localization of such photosensitizers (in particular AlPcS2a) in endocytic vesicles and their subsequent ability to induce a release of the contents of these vesicles - including externally added macromolecules - into the cytosol is the basis for a recent method for macromolecule activation, named photochemical internalization (PCI).

OC315

Reflectance of skin in the UV region

Johan Moan[1], Asta Juzeniene[1], Kristian P. Nielsen[2]

[1]Department of Radiation Biology, Institute for Cancer Research, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway; [2]Department of Physics and Technology, University of Bergen, Bergen, Norway

We have used a bio-optical model of human skin and an advanced radiative transfer model to simulate the penetration of solar radiation into tissue, as well as its reflection. Furthermore, we have measured reflectance spectra, and used the measurements to retrieve the values of important physical and physiological parameters. Surprisingly, dark skin reflects more UV radiation, due to scattering and absorption phenomena, than white skin. This plays a role for both vitamin D production and erythema generation. This reflectance also augments the protective role of a dark skin colour.

IL316

COST 726: long term changes and climatology of UV radiation over Europe

Alois W. Schmalwieser

University of Veterinary Medicine, Institute of Medical Physics and Biostatistics, Vienna, Austria

The ultraviolet (UV) radiation reaching the ground is only a small portion of the radiation we receive from the sun. Nevertheless, UV radiation has a wide variety of effects on humans and the environment. Studies on the impact of UV radiation require knowledge of UV climatology and changes that have occurred in the past. It would be of special importance having the estimates of average and extreme characteristics of the UV impact on various biological systems (including human beings) as well as doses over different time periods. For this the COST726 action was founded in

2004. The main objective of the Action is to advance the understanding of UV radiation distribution under various meteorological conditions in Europe in order to determine UV radiation climatology and assess UV changes over Europe. Since UV solar radiation plays an important role in many processes in the biosphere, including the influence on human organisms, and may be very harmful if UV exposure exceeds "safe" limits, the knowledge of biologically effective UV radiation doses and their geographical distribution and climatology in Europe is crucial. To achieve its general objective, the Action has the following practical objectives: (i) to make an inventory of available solar radiation data sets, including UV data, spectral and broadband, ancillary data (ozone, clouds, sunshine etc.) and available satellite data, (ii) to advance the understanding of UV reconstruction models for the calculations of UV climatology and assessment of UV changes, (iii) to advance the understanding of biological UV radiation climatology and changes in Europe, (iv) to advance the understanding of UV influence on ecosystem, both on the basis of climatology and changes of selected effective UV radiation doses in Europe, (v) to use the advanced knowledge under the points above, in order to elaborate a comprehensive analysis and information basis, addressed to beneficiaries, (vi) Additionally, special attention should be paid to application of QC/QA procedures for the UV measurements with broadband instruments. To get homogeneity of the broadband data, an additional objective is to create a European reference group of broadband radiometers.

The major benefit of the Action will be a climatology of UV radiation and of selected biologically effective UV radiation doses across Europe. The progress of the Action and the outcome is presented at: www.cost726.org

IL317

Quality of UV measurements

Mario Blumthaler

Innsbruck Medical University, Division for Biomedical Physics, Innsbruck, Austria

Measurements of solar UV irradiance are carried out routinely in many countries in order to inform the public about the actual levels of radiant exposure by harmful UV radiation. In almost all of these networks the final product is the UV-Index, which is a measure of the erythemally weighted UV irradiance, scaled to a unitless number, which reaches in midlatitudes normally values up to 10. The quality of these measurements depends on the type of detectors used and on the efforts in QA/QC for the measurements and for the data interpretation. Highest quality of the measurements can be achieved by using spectroradiometers, however, the costs for acquisition and maintenance of these systems are the highest. The commonly used broadband detectors have a spectral sensitivity adapted to the erythema action spectrum. They are relatively cheap, but they need a considerable amount of maintenance to keep the overall uncertainty below 10%. The specific problems of both types of detectors and the best practice for achieving good quality of the measurements will be discussed.

IL318

Modeling UV radiation in the past: achievements and limitations

Peter Koepke[1]*, Jean Verdebout*[2]

[1]*Meteorological Institute, Universitiy of Munich, Muenchen, Germany;* [2]*Joint Research Center, Ispra, VA, Italy*

To get values of UV radiation for times and places without measurements, modeling is necessary. To test the availability of models and input parameters, which are able to describe the relevant atmospheric properties, a modeling exercise has been made in COST 726 by modeling daily doses for two years and 4 stations in Europe. The result is that different high quality radiation transfer models are available. The critical point is the quality and availability of the input data: Ozone, albedo, aerosol and clouds. Cloudiness, due to its high variability in space and time, is the parameter resulting in highest uncertainty in UV-doses. The best way to take clouds into account is the use of cloud modification factors that are derived from measurements of global radiation in the solar spectral range. The UV daily doses, reconstructed at stations with such cloud information, are then spatially interpolated to generate European maps of the monthly averaged daily dose. The interpolation technique is using climatological averages and spatial correlation coefficients drawn from a spatial highly resolved 20 years UV radiation climatology, which is derived by use of satellite data.

IL319

The role of action spectra in determining the biologically effective UV radiation

Gaetano Zipoli, Daniele Grifoni

Institute of Biometeorology, National Research Council, Florence, Italy

The spectrum of solar UV radiation reaching the ground is influenced by the status and the composition of the atmosphere as well as by the optical pathway of radiation through the atmosphere, varying with solar elevation. Changes in the solar elevation angle are responsible not only of changes in the UV irradiance at ground but also in its spectral composition; in fact at low solar elevation angles the UV-B component is much more attenuated by the atmospheric components respect to UV-A. The effectiveness of UV radiation in producing a defined effect on living organisms varies with wavelengths. This is the concept of *action spectrum.* Action spectra are influenced by several factors and can be affected by approximations and uncertainties that may not be accepted any longer when new knowledge are gained: in ther words action spectra may be subjected to modifications and improvement with time. As an example of possible changes in action spectrum we will consider the differences in the UV_{BE} obtained using two different action spectra for plant damage: the so called “generalized plant” action spectrum obtained from Caldwell et al. (1971) and the “growth response of plants” from Flint and Caldwell (2003). The major difference between the two spectra concerns the fact the later takes in some consideration also the contribution of the UV-A band while the first just the UVB band. The results obtained in several experiments aimed to describe the effects of varying UV irradiance due to climate change may be re-evaluated according to the fact that the effects described may be attributed to different doses respect to that exected on the base of the experimental design. Considering experiments with supplementation of lamps irradiance to solar light, the UV_{BE} percentage in enhancement may change dramatically when ”generalized plant” action spectrum was used compared to that obtained using the “growth response of plants” one. The choice of the proper action spectrum may have relevant effects also from a bio-climatological point of view. For each site daily UV_{BE} computed according to “growth response of plants” is much higher compared to the one computed according to the ”generalized plant” action spectrum. Moreover the absolute daily values of UV_{BE} of a southern site is obviously higher compared to a northern one but the differences between the two location are much higher (more than 4 times) using “growth response of plants” action spectrum compared to the “generalized plant” one The bio-climatological implications of these considerations will be discussed in this presentation.

OC320

Exploring the details of UV irradiances, human exposure and dosimetry

Richard Kift, Liam McNulty, Lucy Bunhill, Marie Durkin, Donald Allan, Jacqueline Berry, Lesley E. Rhodes, Ann R. Webb

University of Manchester, Manchester, United Kingdom

Measurements, past reconstructions and future scenarios of UV radiation climates focus on the irradiance incident on a horizontal surface. This is not the usual receiving surface relevant to photobiological effects, particularly when considering humans who have the ability to further moderate their exposure through elective behaviour. Seasonal UV exposures of a population of 125 healthy adults in the Manchester/Salford area are being assessed as part of a study on sun exposure and vitamin D status funded by Cancer Research-UK. Volunteers wear polysulphone film badges for periods of one week in spring, autumn and winter, and 2 weeks in summer. A subset (about 10%) of volunteers also wear recording dosimeters, developed in Manchester, which record exposure with a timestamp every 2 min. Standard UV irradiance measurements (on a flat, horizontal, unshaded surface) are monitored in central Manchester. Volunteers also complete an exposure chart detailing their exposure periods, plus clothing worn and sunscreen use that also affect the biological endpoint of vitamin D status. Here we examine the degree of correlation between the different methods of assessing sun exposure: monitoring + exposure chart, polysulphone dosimeter, and recording dosimeter.

OC321

Reconstructed long-term erythemal irradiance over Europe from measurements of solar irradiance and total ozone

Andreas Kazantzidis[1], Alkiviadis Bais[1], Peter Den Outer[2], Harry Slaper[2], Tapani Koskela[3], Uwe Feister[4], M. Woldt[4]

[1]Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]National Institute for Public Health and the Environment, RIVM, Bilthoven, The Netherlands; [3]Finnish Meteorological Institute, Jokioinen, Finland; [4]Deutscher Wetterdienst, DWD, Lindenberg, Germany

The decrease in stratospheric ozone observed during the 1980s and 1990s and the possible relationship with the observed increase of the ultraviolet (UV) radiation in the biosphere has been extensively discussed in relevant studies. Lack of long-term measurements of surface UV radiation and the influence of clouds, aerosols and surface reflectivity on the transmittance of UV radiation through the atmosphere impose difficulties in the detection of unambiguous trends. Recently, various methods for the reconstruction of UV radiation levels in the past have been proposed. In most of these studies, measurements of total ozone and empirical or model derived relations for the impact of clouds and surface albedo on UV transmittance have been used.

In this study, a method for estimating daily erythemal (CIE) UV doses using measurements of total ozone and solar irradiance is presented and applied to data recorded at four European stations, representing different geographical and environmental conditions. Measurements of shortwave and erythemal solar irradiance between 1999 and 2004 were used to determine the effect of clouds. The method is then tested, by comparison to measurements conducted in two periods, 1992-1999 and 2005-2006. Finally, the method is applied to solar irradiance and total ozone data for previous years and the changes in the reconstructed UV irradiance time series are presented.

OC322

Photoprotection and skin cancer prevention in the Czech caucasion population

Michal Janouch[1], Karel Ettler[2]

[1]Solar and Ozone Observatory of the Czech Hydrometeorological Institute, Hradec Kralove, Czech Republic; [2]Department of Dermatology, Charles University, Hradec Kralove, Czech Republic

The Czech Hydrometeorological Institute (CHMI) is responsible for monitoring of the UV solar radiation in the Czech Republic. The UV monitoring program is carried out at the Solar and Ozone Observatory in Hradec Králové (SOO-HK). Results of observations are deposited in the UV database of CHMI and also submitted to the European UV Data-center in Helsinki. Systematic daily UV measurements have been performed since 1994 at SOO-HK with the Brewer spectrophotometers (B098 and B184). The erythemally effective UV radiation is measured by broad-band UV Biometers in CR at the SOO-HK, Observatory Košetice (GAW station) and Labska station in the Giant Mountains. The instruments are regularly calibrated towards international standards and produce the first-class quality data. The B098 and B184 are the only UV spectroradiometers operated in CR for measurements of spectral intensities of UV radiation and thus serve as a reference instruments for the UV public warning system in the country.

The use of results of our UV measurements (i.g. UV index) seems to be very important. We cooperate very closely with the Dept. of Dermatology, Charles University, Hradec Králové on this field. Every year (since 2002) the Euro Melanoma Day (usually the second Monday of May) is presented in media as a day of open dermatological offices for everybody who is suffering from moles and skin dark tumors. In terms of this Day a lot of articles and news are published in newspapers and journals focused on photoprotection. Minimal erythema doses (MED) of caucasian Czech population (phototype I – IV) has been established by cooperating dermatologists. The time till erythema occurrence could be calculated from MED and UV intensity. This time has a key importance for a choice of Sun Protection Factor (SPF) of sunscreens. We suggest our activity as a important contribution to skin cancer prevention.

OC323

UVBE maps for Poland – preliminary results for selected action spectra

Zenobia Litynska[1], Aleksander Curylo[1], Bozena Lapeta[2], Julita Biszczuk[1], Janusz Krzyscin[3], Barbara Bogdanska[4], Jakub Walawender[2]

[1]Centre of Aerology, IMWM, Legionowo, Poland; [2]Satellite Research Department, IMWM, Krakow, Poland; [3]Institute of Geophysics, Polish Academy of Science, Warsaw, Poland; [4]Meteorological Centre, IMWM, Warsaw, Poland

UV solar radiation plays an important role in many processes in the biosphere and may be very harmful if UV exposure exceeds "safe" limits. Therefore, the knowledge of UV biologically effective (UVBE) radiation doses and their geographical distribution is crucial for the population. The most important source of the information about UV radiation is the ground stations network. However, for the most countries, the spatial distribution of the stations does not allow for creating of UV maps. In Poland, the UV broadband measurements have been performed at three stations since 1994. On the other hand, the global solar radiation network includes c.a. 20 stations. Therefore, UV reconstruction models are used to calculate the UV radiation on this denser global solar radiation network.

The works aimed at creating the monthly mean UV maps for different action spectra for Poland. The UV reconstruction model developed at IMWM and tested in the COST-726 modelling exercise was used. The UV calculations were performed on the base of the daily global solar radiation and ancillary data from the period of 1991-2001, for summer. Selected biological effective action spectra functions were then applied. Next, the monthly mean

UVBE values were calculated and were interpolated with the use of kriging interpolation algorithm. The obtained UVBE maps will be presented.

IL324
Skin phototesting
Brian Diffey
School of Clinical and Laboratory Sciences, University of Newcastle, Newcastle, UK
The investigation of photosensitive diseases will often involve phototesting. The object of this procedure is to reproduce the lesions of a disease so as to confirm the diagnosis, and/or to determine the action spectrum, which can be useful either as a means of identifying a potential photosensitizer or enabling suitable preventative measures to be taken e.g. the choice of a topical sunscreen with an appropriate absorption profile. Professor Ian Magnus was one of the first to realise the usefulness of action spectroscopy of the skin when almost 50 years ago he was able to associate the pronounced response for erythema and wealing at 400 nm in a subject with porphyria cutanea tarda with the strong absorption by porphyrin in this spectral region.
To maximise the clinical usefulness of phototesting, careful attention needs to be paid to the physical characteristics associated with the procedure. These include knowledge of the spectral power distribution of radiation source used in the procedure and an accurate delivery of radiation dose.

IL325
The porphyrias
Gillian M Murphy
National Photodermatology Unit, Beaumont & Mater Hospitals, Dublin, Ireland
The porphyrias are a consequence of deficient activity of the enzymes which control Haem biosynthesis. Since the first mention of porphyria in the literature by Scherer in 1841 much is now understood. Acute porphyria was described by Stokvis in 1889, congenital erythropoietic porphyria in 1911 by Günther. By the 1950s acute intermittent porphyria, variegate porphyria and endemic porphyria cutanea tarda (PCT) were well decscribed. However in 1961 a new form of porphyria, erythropoietic protoporphyria (EPP), now known to be the third commonest porphyria, was clearly described by Ian Magnus. Since then the genetic basis of this whole group of disorders has been elucidated. Susceptibility factors predisposing to these diseases have also been discovered including co-inheritance of genes influencing iron metabolism in the case of PCT and over 70 different ferrochelatase (FECH) mutations have been identified in EPP patients. EPP occurs in most families by the combination of a disabling FECH mutation and a common, intronic polymorphism (IVS3-48T/C) affecting the other FECH allele, which leads to an aberrantly spliced mRNA then easily degraded by a nonsense-mediated decay mechanism, decreasing the level of FECH mRNA. Without this polymorphism, EPP is associated with inheritance of two FECH mutations and autosomal recessive inheritance but only rarely. Thus EPP is mainly an autosomal dominant disease at the molecular level, but inheritance of two alleles causing reduced FECH activity is required for sufficient erythroid accumulation of protoporphyrin to cause symptoms.
Protoporphyric liver disease is believed to result from delivery of excess rotoporphyrin to the liver. Null mutations, result in a truncated protein, when heteroallelic with the IVS3-48T/C polymorphism, have been associated with risk for EPP-related liver disease. Likewise in all of the other forms of porphyria detailed study of the genetic mutations has lead to significant understanding of how these diseases come about. Late onset of porphyries usually seen in childhood may be a consequence of clonal expansion of malignant cells carrying acquired mutations. Cure for the erythropoietic porphyrias and marked alleviation of symptoms may now be achieved by bone marrow transplantation and liver transplantation respectively.

IL326
The immunological photodermatoses
John L M Hawk
St John's Institute of Dermatology, London, UK
The five probably immunological photodermatoses comprise firstly polymorphic light eruption (PLE), actinic prurigo (AP) and hydroa vacciniforme (HV). These three are all arguably delayed type hypersensitivity papular or vesicular eruptions, relatively convincingly so in the cases of PLE and AP but much less so for HV. They occur within hours of sun exposure and last for days to weeks, apparently as responses against ultraviolet radiation (UVR)-induced cutaneous auto-antigen, perhaps on clinical grounds mostly dermal in location. Chronic actinic dermatitis seems to be a similar but persistent reaction of superficial eczematous nature, perhaps again on clinical grounds epidermal in site, and also convincingly a delayed type hypersensitivity response, while solar urticaria appears almost definitely to be a rapid onset, short-lived immediate type hypersensitivity reaction against presumed dermal UVR-induced cutaneous auto-antigen. All these disorders except HV have effective or very effective therapies, whether sunscreen prophylaxis or immunosuppressive topical, oral or phototherapy. The late Professor Ian Magnus of the St John's Institute of Dermatology, London, developed the first major research unit investigating the nature, causation and particularly treatment of all these disorders, the last investigations being carefully controlled trials that often debunked the claims of earlier anecdotal therapeutic reports. He also clarified the nature of the diseases as then known in his seminal book publication, Derrmatological Photobiology. Thereafter, his students eagerly took up his unfinished work, particularly concerning the pathogenesis of the disorders, and advanced it significantly from his exceptionally sound and brilliantly conceived beginnings.

OC327
Photosensitive psoriasis: clinical characteristics and potential role of memory effector T lymphocytes in the pathogenesis
Kirsty J Rutter[1], *Lindsey F Cotterell*[1], *Margaret M Brownrigg*[1], *Thomas Brenn*[2], *Christopher EM Griffiths*[1], *Rachel EB Watson*[1], *Lesley E Rhodes*[1]
[1]*Photobiology Unit, Dermatological Sciences, University of Manchester, Manchester, United Kingdom;* [2]*Department of Pathology, Salford Royal Hospitals Foundation Trust, Manchester, UK*
A minority of patients with chronic plaque psoriasis have severely photosensitive psoriasis (PP), with pronounced symptoms in summer months and mild or absent disease in the winter, though the underlying pathomechanism is poorly understood. Our aims were (i) to perform a detailed clinical characterisation of patients with PP and (ii) to explore the underying pathomechanisms in a randomised study.
Patients (n=20) with PP were predominantly female (19F:1M), with mean (range)age at onset of psoriasis 11(2-24)y. A positive family history of psoriasis was reported by 13 patients, 7 in 1st degree relatives. Onset of photosensitivity was synchronous with onset of psoriasis in 11 patients and developed later in the remainder. Broadband UVA provocation produced a clinical response in 17/20 patients, comprising erythema +/- scaling plaques. The early histological response to photoprovocation was then examined in a randomised study of patients with PP (n=10) vs. non-photosensitive psoriasis (NP; n=10) vs healthy volunteers (C; n=12). Low dose broadband UVA (20J/cm^2, 320-400 nm) was given to ventral forearm skin on 3 consecutive days. Skin biopsies were taken 0h and 6h, 24h and 7d post provocation. Samples were assessed

immunohistochemically for $CD4^+$, $CD8^+$ and $CD45RO^+$ T-lymphocyte subsets, and epidermal thickness quantified by image analysis. Histological features of lymphocytic infiltration and epidermal thickening occurred in 4/10 PP patients, but in no other subjects. This PP subgroup (PPH; n=4) was analysed separately from those with no histological change (PPN; n=6). Epidermal thickening occurred at 7d (mean ± SE µm, C: 53.4 ± 2.6; NP: 64.4 ± 3.6; PPN: 54.3 ± 5.5; PPH: 72.3 ± 9, p<0.05 for PPH vs. C). $CD8^+$ cells were increased in PPH, and maximal 24h post-UVA (cells/hpf, 0h: 12.1 ± 4.8; 6h: 9.0 ± 4; 24h: 18.2 ± 4.7; 7d: 13.2 ± 3.0; p<0.001 for PPH vs. C). $CD45RO^+$ cell expression mirrored $CD8^+$ (0h: 9.1 ± 2.4; 6h: 11.3 ± 4.1; 24h: 20.3 ± 11.8; 7d: 8.3 ±1.3, p<0.001 for PPH vs. C).

We have defined a group of severely photosensitive psoriasis patients whose photosensitivity is associated with: UVA provocation, pronounced female predominance, early onset and positive family history. Histological features consistent with psoriasis are rapidly photoprovoked in some individuals, with a potential role for memory effector T cells in the early phase.

This research was funded by the Psoriasis Association, UK.

OC328

High levels of anxiety and depression related to their condition reported in a hospital based PLE population

Tsui C. Ling[1], H.L. Richards[2], M. Brownrigg[1], Rebecca Brooke[1], K. Huber[1], N.K. Gibbs[1], L.E. Rhodes[1]

[1]Photobiology Unit, Dermatological Sciences, University of Manchester, Manchester, United Kingdom; [2]Division of Clinical Psychology, University of Manchester, Manchester, United Kingdom

Photosensitivity disorders may cause psychological distress, but this area is under-explored. We have examined for psychological distress in patients with polymorphic light eruption (PLE), and correlated this with indicators of life quality and clinical severity of condition.

Seventy patients attending a photobiology unit for diagnostic evaluation self-completed a range of questionnaires during the summer months. These assessed levels of anxiety and depression (Hospital Anxiety and Depression Scale; HADS), Fear of Negative Evaluation (FNE) and beliefs about their condition (Illness-Perceptions Questionnaire-Revised; IPQ-R), in addition to quality of life (Dermatology Life Quality Index; DLQI) and clinical pattern of the condition. Participants were mean age 42.5 (SD 12) y, 81% female, and had a mean age of onset of 27 (SD 14) y. Mean DLQI score was 7.6 (SD 7). High levels of self-reported distress were identified, with 25.7% and 7.1% of patients scoring on the HADS as probable cases for anxiety and depression respectively, and 40% of individuals with scores on the FNE indicative of high social anxiety. Those with a younger age of onset of PLE reported higher levels of anxiety (r =-0.3, p<0.05). Involvement of the face and neck were associated with significantly higher DLQI scores (t's>2.5, p's<0.02) and higher levels of depression (t's>2.9, p's<0.01). Annual duration of involvement of PLE symptoms (number of months affected/year) was associated with anxiety level and stronger belief that their condition would be prolonged ($F_{2,69}$=2.83, p<0.05). Moreover, patients whose rash took longer to resolve had higher DLQI scores ($F_{2,69}$=4.86, p=0.01), higher levels of depression ($F_{2,69}$=3.52, p<0.05) and believed the consequences of their condition were greater ($F_{2,69}$=4.34, p<0.01). Stepwise multiple regression indicated that depression accounted for 36% of the variance in DLQI scores, while the clinical variable of number of months affected accounted for 4% of the variance.

This study reveals very high levels of psychological distress related to PLE in a hospital-based population. Patients, particularly those with indicators of more severe disease, i.e. early onset, face and neck involvement, and longer duration of rash, might benefit from psychological management to assist adjustment to their condition.

This work was financed by EU Framework V Programme, project no. QLK4-CT01-0015.

OC329

The effect of capsaicin-induced neuropeptide depletion on the induction of polymorphous light eruption by solar simulated light

Franz J Legat, Agnes Bretterklieber, Manuela Haar, Goran Sepic, Angelika Hofer, Alexandra Wackernagel, Franz Quehenberger, Helmut Kerl, Peter Wolf

Medical University Graz, Department of Dermatology, Research Unit for Photodermatology, Graz, Austria

Polymorphous light eruption (PLE) is the most common idiopathic photodermatosis. While present hypotheses about the pathogenesis of PLE comprise i) an impaired infiltration of neutrophils into the skin after UVR exposure, ii) a delayed-type hypersensitivity reaction to yet unknown putative photoallergens, as well as iii) a failure of normal UVR-induced immunosuppression, the cause of the disease remains unknown. Neuropeptides from cutaneous sensory nerve fibers have been shown to play a role in local and systemic UVR-induced immunosuppression. Thus, in this study we investigated whether capsaicin-induced depletion of neuropeptides from cutaneous sensory nerve fibers would affect the induction of PLE by solar simulated light.

Fifteen patients (12F/3M; median age 38 years, range 22-65 years) administered a capsaicin 0.05% cream or its vehicle, respectively, 4 times per day for 14 days to one PLE-prone skin area on each of two contralateral body sides. Within the contralateral pretreated skin areas, then test fields of 5x5cm were irradiated with solar-simulated light (SSR, Oriel® 1 kW Xenon arc solar simulator) once per day for 4 consecutive days. SSR-dose was 70% of the individual minimal erythema dose (MED) at the start. On each consecutive day, the SSR dose was increased by up to 40% according to the erythema reaction within the test fields after previous SSR exposure(s). Both test fields always received equal SSR doses. At 24, 48, 72, 96, and 168h after the first SSR exposure, test fields were evaluated for the induction of PLE lesions and erythema, using clinical scores, as well as for itch sensation, using a visual analogue scale (VAS) from 0 to a maximum of 10.

Twelve of 15 patients developed PLE lesions within the test fields during the observation period of 7 days. There was no significant difference in the clinical scores for PLE lesions or erythema, or in VAS values for itch sensation comparing the contralateral test fields pretreated with capsaicin 0.05% cream or vehicle, respectively. We conclude that neuropeptides may not play a significant role in the induction of the clinical signs for PLE by solar simulated irradiation, at least based on the experimental depletion conditions we had used in this study.

IL330

***In vitro* phototoxicity testing: a task involving multiple endpoints**

Laurent Marrot, Jean-Philippe Belaidi, Christophe Jones, Anita Labarussiat, Philippe Perez, Jean-Roch Meunier

Phototoxicity Unit, Safety Research Dpt, L'OREAL, Aulnay-Sous-Bois, France

Today's lifestyle is often associated with frequent exposure to sunlight. In this context, some chemicals used in drugs, cosmetics or food sometimes produce adverse biological effects when irradiated. In general, they only induce slight photo irritancy, but sometimes exaggerated erythema can be observed. At worst, they can produce DNA damage in addition to those due to UV radiation itself, increasing the risk of skin cancer. There is thus a need for appropriate methodologies aiming at obtaining relevant data at the molecular and cellular levels for such compounds. For ethical and practical reasons, *in vitro* models have got an increasing importance in safety screening. Here, we propose a strategy based on complementary tests for assessment of the photocytotoxic and photogenotoxic potentials. First, the use of a light source that mimics environmental solar UV is presented as an essential requirement: in this regard, solar simulators appear to be most

appropriate available equipment. Then, complementary methods with increasing complexity are implemented in order to get a rational evaluation of the phototoxic risk: (i) supercoiled circular DNA for *in tubo* assessment of photoreactivity, (ii) yeast *Saccharomyces cerevisiae* for evaluation of photocytotoxicity and photomutagenicity, (iii) cultured human skin cells where photogenotoxicity can be detected using the comet assay (iiii) and finally reconstructed skin where cell death as well as DNA damage can be measured after either systemic or topical application of the studied chemical. Such a strategy is particularly well adapted to rigorously ensure the safety of products likely to undergo environmental sunlight exposure.

IL331

Dermatological overview of phototoxic reactions: a mechanistic approach

Paulo Leal Filipe[1], Patrice Morlière[2], René Santus[3]

[1]University of Lisbon, Department of Dermatology, 1649-028 Lisbon, Portugal; [2]INSERM ERI12, Université de Picardie Jules-Verne, CHU Amiens-Nord, F-80054 Amiens, France; [3]INSERM U697, Institut de Recherche sur la Peau, Hôpital Saint-Louis, F-75475 Paris cedex 10, Paris, France

Drug-induced photosensitivity refers to the development of cutaneous disease as a result of the combined effects of a chemical and light. Exposure to either the chemical or the light alone is not sufficient to induce the disease; however, when photoactivation of the chemical occurs, one or more cutaneous manifestations may arise. These mainly include phototoxic and photoallergic reactions. Photosensitivity reactions may result from systemic medications and topically applied compounds. Wavelengths within the UV-A (320-400 nm) range and, for certain compounds, within the visible range, are more likely to cause drug-induced photosensitivity reactions, although occasionally UV-B (290-320 nm) can also be responsible for such effects. In patients who present with photosensitivity, it is often difficult to differentiate phototoxic from photoallergic reactions. However, they have a number of distinguishing characteristics. Photoallergic reactions resemble allergic contact dermatitis, with a distribution limited to sun-exposed areas of the body. However, when the reactions are severe or prolonged, they may extend into covered areas of skin. Photoallergic reactions develop in only a minority of individuals exposed to the compound and light; they are less prevalent than phototoxic skin reactions. The amount of drug required to elicit photoallergic reactions is considerably smaller than that required for phototoxic reactions. Phototoxic reactions develop in most individuals if they are exposed to sufficient amounts of light and drug. Moreover, photoallergic reactions are a form of cell-mediated immunity; their onset often is delayed by as long as 24-72 hours after exposure to the drug and light. By contrast, phototoxic responses often occur within minutes or hours of light exposure. Typically, they appear as an exaggerated sunburn response. Phototoxic reactions result from direct damage to tissue caused by a photoactivated compound. Phototoxic reactions occur because of the damaging effects of light-activated compounds on cell membranes and, in some instances, DNA. By contrast, photoallergic reactions are cell-mediated immune responses to a light-activated compound. Many compounds have the potential to cause phototoxicity. Most have at least one resonating double bond or an aromatic ring that can absorb radiant energy. Most compounds are activated by wavelengths within the UV-A (320-400 nm) range, although some compounds have a peak absorption within the UV-B or visible range. In most instances, photoactivation of a compound or metabolites results in the excitation of electrons from the stable singlet state to an excited triplet state. As excited-state electrons return to a more stable configuration, they transfer their energy to oxygen, leading to the formation of reactive oxygen intermediates. Reactive oxygen intermediates such as hydroxyl, oxygen singlet, superoxide anion, and hydrogen peroxide damage cell membranes and DNA. Signal transduction pathways that lead to the production of proinflammatory cytokines and arachidonic acid metabolites are also activated. The result is an inflammatory response that has the clinical appearance of an exaggerated sunburn reaction. In order to understand the role of oxidative stress in phototoxic reactions, we study the protection of some antioxidant flavonoids against the photo-oxidative stress in human skin fibroblasts and keratinocytes cultures induced by photosensitised cyamemazine (a phototoxic neuroleptic).

IL332

Effects of UVB light on antiinflammatory corticosteroids in different experimental models

Sergio Caffieri, Giorgia Miolo

Department of Pharmaceutical Sciences, University of Padova, Padova, Italy

Glucocorticosteroids, natural hormones derived from α-pregnane, are potent therapeutic agents for the treatment of a broad range of inflammatory diseases. Semisynthetic derivatives are widely used systemically mainly for the treatment of rheumatoid diseases and allergic manifestations, and many of them are effective by topical use in dermatoses and other skin diseases. This class of drugs is sensitive to UV radiation. They are a typical example of bichromophoric moiety: all possess an aliphatic ketone in the side chain linked to position 17 of the D ring, which absorbs UVB light. Ring A bears a keto group that is conjugated with either one or two double bonds, depending on the specific drug. In the former case (*i.e.*, hydrocortisone) the chromophore is mainly sensitive to UVB. In the latter (*i.e.*, betamethasone, fluocinolone, triamcinolone, flumethasone) both UVA and UVB effectively induce photolysis. The photodegradation of these drugs was studied *in vitro* (in the solid state, in organic and aqueous solutions, in commercial formulations) and *ex vivo* (in the pig skin) models. Both primary photoprocesses, cyclohexadienone 'lumi' rearrangement (under UV-A) and C-20 ketone homolysis (under UV-B) occur and the main photoproducts formed have been isolated and characterized. Any modification of the structure of corticosteroids, in particular the loss of the side-chain, has profound effect on their anti-inflammatory activity.

The drugs are also able to photoreact with biological substrates mainly through radical intermediates and one of them (betamethasone) shows phototoxic effects both *ex vivo* and *in vivo* (mice) too. All these results suggest to protect these drugs from light not only during storage but also after *in vivo* administration to avoid loss of therapeutic activity and potential phototoxic reactions.

IL333

Phototoxicity of tricyclic antidepressants

Carmelo García[1], Rolando Oyola[1], Luis E. Piñero[1], Rafael Arce[2]

[1]University of PR - Humacao, Humacao, Puerto Rico; [2]University of PR - Rio Piedras, Rio Piedras, Puerto Rico

Neuroleptic drugs are used primarily for the treatment of schizophrenia, mania, anxiety, dementia, and drug abuse. The major used neuroleptics belong to the general tricyclic antidepressants (TCA) family (phenothiazines, dibenzazepines, and dibenzodiazepines). Most of the derivatives of these drugs do also produce serious side effects, including tardative dyskenia, parkinsonism, menstrual irregularities, hypertension, allergy, and photosensitization. Small changes in the structure of the derivatives change the mode of action of the drugs, the potency and the spectrum and severity of the side effects. The molecular photochemical mechanisms for their photosensitizing ability are still unknown, even though they are widely used to treat annually thousands of psychiatric patients. Laser flash and steady-state photolysis studies of some TCAs showed that the triplet state of the halogenated derivatives (^{3}TCA*) are efficiently quenched by

protons in the solution. The effectiveness of this quenching is very sensitive to the structure of the drug and seems to correlate well with their phototoxicity. To better understand the involvement of the excited specie in the phototoxicity mechanism, the photophysical and photochemical properties of several series of tricyclic neuroleptics were measured. Absorption, luminescence, and laser flash photolysis were used to characterize their short-lived transients, especially of the cation radical and the triplet. The results indicate that, besides the intrinsic properties of the corresponding ^{3}TCA*, data on its interaction with some membrane components is required to fully explain the large differences in phototoxicity of drugs with similar structure and properties.

Financial support to this work was provided by the NIH-SCORE Grant to the University of Puerto Rico at Humacao.

OC334

Site-selected photoelectron transfer and covalent binding in Nalidixic Acid:HSA complexes

Sandra Monti, Ilse Manet, Francesco Manoli, Massimo Capobianco

CNR-Istituto per la Sintesi Organica e la Fotoreattività, Bologna, Italy

Photoallergic reactions induced by drugs were attributed to modification of proteins consisting in photoinduced formation of covalent adducts with antigenic power. The molecular mechanisms of such photoprocesses were mainly studied with non steroidal anti-inflammatory drugs (NSAID) of the arylpropionic acids family, in matrices of serum albumins (SA) taken as model proteins. Photoallergy was found to be associated also with the therapeutical use of fluoroquinolones. Thus, in order to gain more insight into the photodamaging potential of the 4-quinolone nucleus toward proteins, we undertook the study of complexation and photoreactivity of the antibacterial naphtyridone, nalidixic acid (NA), within human and bovine SA (HSA and BSA). The most stable NA:SA complexes were characterized as regards their stoichiometry, stability constants, UV absorption coefficients, circular dichroism and fluorescence parameters. Laser flash photolysis evidenced two site-selected electron transfer processes, involving photoexcited NA and specific aminoacids of the protein, leading to radical pairs. Kinetic and product studies revealed the formation of covalent drug-protein adducts via radical-radical combination. We believe this study can contribute to the understanding of the mechanism of protein modification by photoexcited 4-quinolones with direct relevance to the control of side photoeffects by these antibacterials. Further this investigation illustrates nicely the potential of combined application of multiple spectroscopic, photochemical and analytical techniques to gain insights into site selected reactions of drugs with proteins.

OC335

Primary photoprocesses in some photosensitive drugs studied using fast reaction kinetic techniques

Suppiah Navaratnam[1,2]

[1]Biosciences Institute, University of Salford, Salford, UK; [2]STFC Daresbry Laboratory, Warrington, UK

Drug induced photosensitization of the skin and eye is a problem that is increasingly attracting the attention of the clinicians, pharmaceutical industry and regulatory authorities. Although first recognised at the beginning of the century, adverse skin reactions due to sulphonamides in the 1940s, chlorpromazine and tertracyclines in the fifties non-steroidal anti-inflammatories in the eighties and fluoroquinolones in the nineties increased the awareness on useful drugs that might cause problems due to photosensitization. Clinically reported photosensitised adverse effects of these drugs incidents include cataract of the lens, skin eruptions, and onycholysis where the nail plate separates from the nail bed.

The mechanism underlying such effect, in most cases, is not completely understood. A number of in-vitro and in-vivo experiments have been carried out in order to understand the mechanism(s) of such reactions. However, most of these studies monitored the effects well removed from the initial act of light absorption by the drug. A detailed knowledge of the photochemistry and photophysics of the drugs under conditions representative of their actual use will aid the understanding of the mechanism of the biological effect. To achieve this goal it is necessary to obtain quantitative data on the types and reactivity of the excited states and free radicals of the drug formed on the absorption of light energy.

We have investigated a number of classes of such drugs and in all cases, the triplet state was formed with relatively high quantum yields and reacted with molecular oxygen, almost quantitatively, to form excited singlet oxygen. Bond cleavage from both excited singlet and triplet states were observed, in some cases, resulting in the formation of free radicals followed by photoproducts. In some cases, both mono- and bi- photoionisation was observed. Most of the free radicals formed were found to react with biological substrate, such as amino acids and proteins as well as with DNA.

OC336

Photodehalogenation of fluoroquinolones

M. Consuelo Cuquerella[1], Francisco Boscá[2], Miguel A. Miranda[2]

[1]Universidad de Valencia/Instituto de Ciencia Molecular, Paterna, Valencia, Spain; [2]Instituto de Tecnología Química/Universidad Politécnica de Valencia, Valencia, Valencia, Spain

Fluoroquinolones (FQ) are widely used antibacterial agents that exhibit photosensitivity side effects. Some of them have even been demonstrated to be photocarcinogenic and photomutagenic. Photochemistry and photophysics of FQ has been studied and two different reaction pathways have been described depending on the substitution in position 6 and 8. In the case of 6-fluoroquinolones their photodehalogenation in aqueous solutions leads to the formation of 6-hydroxyquinolones by photosubstitution of fluoride anion in neutral media. A direct attack of OH^- to the excited triplet state has been proved by steady state and time resolved studies. However, when a halogen is present in the position 8 of the main ring of FQ, the photo-process is produced by the mediation of a detectable transient species with an absorption maximum around 490 nm that it can be assigned to an aryl cation. In this case, the reaction is completed by attack of nucleophiles present in the aqueous solution and by insertion into the neighboring C-H bond of the N-ethyl group.

OC337

Mechanisms of the selective photodynamic efficiency of the hexyl-aminolevulinate (h-ALA)-protoporphyrine IX (PpIX) in the treatment of the bladder cancer

Berrahmoune Saoussen[1], El Khatib Sami[1], Brie David[2], Fotinos Nicolas[3], Guedenet Jean-Claude[4], Bezdetnaya Lina[1], Guillemin François[1], D'Hallewin Marie-Ange[1]

[1]Centre Alexis Vautrin Laboratory CRAN CNRS UMR 7039 Nancy University, Vandœuvre-lès-Nancy, France; [2]CRAN UMR 7039 Faculté des sciences et techniques, Vandœuvre-lès-Nancy, France; [3]Laboratoire de Pharmacie Galénique et de Biopharmacie, Université de Science II, Genève, Suisse; [4]Unité fonctionnelle de Microscopie électronique, CHU de Nancy, Vandœuvre-les-Nancy, France

The goal of this work was first to study the efficiency of the photodynamic therapy in the treatment of the bladder cancer and to explain the mechanisms that govern this efficiency.

In our study we have used Hexvix® or hexylaminolevulinate (hALA), that inside the cells induced PpIX formation. The tumor model orthotopic and syngenic, developed in Female Fischer Rats

344. We have previously shown that rat tumor bladders instilled with 8 mM Hexvix® induced tumor necrosis with intact bladder wall but instillation with 16 mM Hexvix® showed total wall necrosis without impact on the tumor. We have investigated fluorescence spectroscopy *in situ*, photobleanching kinetics *in vivo*, the quantity of PpIX and the formation of different fluorescent porphyrins by HPLC, after both hALA instillation. We have also studied mitochondial damage after PDT by immunofluorescence of beta ATP synthase (a mitochondrial marker) and electronic microscopy on bladder tumors sections at both hALA concentrations.
The same initial fluorescence intensities were achieved at both hALA concentrations. The photobleaching of PpIX in bladder cancer follows mono-exponential kinetics with similar decay constants at 8 mM (K = 0,296, Chi^2 = 0,98) and 16 mM (K = 0,27, Chi^2 = 0,74). One photoproduct, photoprotoporphyrin IX was observed with a fluorescence peak around 670 nm at both concentrations. HPLC indicates that 90 % of the total amounts of fluorescing porphyrin produced was protoporphyrin IX. However the quantity of PpIX formed at 16 mM hALA is 3 times lowers than at 8 mM d'hALA. The results suggest that there are modifications of the metabolic way of PpIX at large Hexvix concentrations. The mitochondrial damage was evaluated by immunohistochemistry at two end points after PDT (0 h and 4 h). At 0 h the same punctiform labelling was observed at both hALA concentrations, similar to controls. However at 4 h the labelling was diffuse cytoplasmic at 8mM hALA contrarily to 16 mM d'hALA where the marking comparable to controls. We suppose that this difference is due to damage of the mitochondria caused by PDT for 8 mM hALA. Further electron microscopic studies of these damages are in progress.

OC338

Effects of hexyl 5-aminolevulinate and light in a rat bladder cancer model

Odrun Gederaas[1], Lise Lyngsnes Randeberg[1], Eivind La Puebla Larsen[1], Astrid Hjelde[1], Carl-Jørgen Arum[1,2], Anders Brunsvik[3], Chun-Mei Zhao[1], Duan Chen[1], Lars Ottar Svaasand[1], Hans E. Krokan[1]
[1]Norwegian University of Science and Technology, Trondheim, Norway; [2]University Hospital, Trondheim, Norway; [3]Dept. Clinical Pharmacology, University Hospital, Trondheim, Norway

Introduction. The purpose of this study was to monitor immediate tissue response, and to provide information for optimizing drug and light doses in a rat model for superficial bladder cancer. The relatively new ester derivate, hexyl 5-aminolevulinate (HAL), was used to stimulate intracellular photosensitizer formation.
Materials and Methods. Female Fisher F344 rats were instilled with AY-27 cancer cells through a catheter following an acid/base wash. On day 14, the animals were catheterized and instilled with HAL (8mM, 1h). Two hours after instillation, the animals received a single light dose (20 J/cm^2) delivered using a fibre optic applicator (200µm, isotropic diffuser tip) inserted through an 18 gauge catheter in the urethra. The applied light source consisted of an Argon-pumped dye-laser centered at 635 nm. The fluence rate used for all treatments was 20mW/cm^2. The same light source was used for the *in vitro* studies on rat bladder carcinoma cells (AY-27) after incubation with HAL (5µM, 3.5 h). Using high pressure liquid chromatography (HPLC), the biosynthesis of different porphyrins was documented. Tissue oxygenation was measured by reflectance spectroscopy prior to and after the light treatment. Fluorescence spectroscopy was used to monitor PpIX fluorescence.
Results and Conclusions. A total light dose of 20 J/cm^2, combined with 8 mM HAL, is adequate to study the efficacy of hexyl-ALA-PDT, when applied on day 14. As revealed by histology, the development of tumour cells was associated with increased rate of the epithelial cell proliferation. Tissue oxygenation was decreased in animals with carcinomas compared to control animals, and PpIX fluorescence in HAL treated animals was partly bleached immediately after light treatment. No PpIX fluorescence could be detected 24 hours after treatment. Monitoring of tissue responses provide important information to optimize physical as well as chemical factors, such as drug and light dose for this treatment modality.

OC339

MAL-PDT of squamous cell carcinoma

Piergiacomo Calzavara-Pinton, Raffaella Sala, Marina Venturini, Giovanni Parrinello, Cristina Zane
University of Brescia, Brescia, Italy

Background: PDT with methyl-MAL is an approved non- invasive treatment option for AK, a precursor of squamous cell carcinoma (SCC).
Patients: A total of 112 biopsy-proven SCC in 55 outpatients were treated.
Interventions: A MAL cream (160 mg/g) was applied for 3 hours prior to illumination from a LED source (wavelength range: 635±18 nm; light dose 37 J/cm^2). Nodular lesions (prominent above skin surface >2mm) were debulked surgically prior to be treated. A second MAL PDT session was given 7 days later.
Results: The overall complete response rates were 73.2 % at 3 months and 53.6 % at two years. Clinical thickness, atypia and lesion depth were significant predictors when using a univariate analysis ($p<0.001$). A multivariate logistic regression model, with robust variance estimation, showed that cell atypia was the only independent predictor of the treatment outcome. The time (mean± SD) to recurrence was 6.55±4.10 months.
The relapse rate at follow-up was influenced by cellular atypia (HR=5.23; 2.12-12.87;p=<0.001) and maximal diameter (HR=1.21;1.03-1.41;p=0.018) of the lesion according to the Cox model with adjustment for correlated data.Local adverse effects consisted of strong erythema followed by erosion or ulceration. Pain was mild to moderate and treatment discontinuation was never required.
The cosmetic outcome was good or excellent in the majority of patients with a very good concordance of evaluation among patients and investigators. The most important predictor for cosmesis was cell atypia.
Conclusion: MAL-PDT may represent a valuable, effective and well tolerated treatment option with a good cosmetic outcome for superficial, well- differentiated (Broders' scores I and II), in situ SCCs. In contrast, its use for superficial SCCs with a microinvasive histological pattern and for nodular, invasive lesions, particularly if poorly differentiated (Broders score III and IV) keratinocytes are present, should be avoided and, if surgery and other invasive procedures are not applicable, other therapeutic alternatives, e.g. radiotherapy or local chemotherapy, should be used.

OC340

Protoporphyrin IX production and photodynamic effects after application 5-aminolevulinic acid or its heptyl ester

Asta Juzeniene[1], Xiao Puzhuoma[2], Johan Moan[1]
[1]Department of Radiation Biology, Institute for Cancer Research, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway; [2]Tibet University, Lhasa, Tibet, People's Republic of China

5-aminolevulinic acid (ALA) is a precursor of heme and is currently being used to induce protoporphyrin IX (PpIX, a photosensitizer) for photodynamic therapy (PDT). Since the efficacy of the treatment is limited by the hydrophilic nature of ALA, chemical modifications, such as etherification with aliphatic alcohols, have been made to induce higher porphyrin production. A new 5-aminolevulinic ester, heptyl 5-aminolevulinate (ALA-heptyl ester), was compared with ALA were for induction of porphyrins in human adenocarcinoma WiDr cells and in Balb/c nude mice. Incubation of WiDr cells with ALA or ALA-heptyl ester result in accumulation of fluorescent porphyrins. The fluorescence

excitation and emission spectra were similar for all studied concentrations of ALA and ALA-heptyl ester. These spectra can be attributed to PpIX. Concentrations higher than 0.01 mM of ALA heptyl ester were toxic to the cells, while 0.01-1 mM of ALA were non-toxic. The fluorescence intensities of PpIX were the similar for both drugs when cells were exposed to 0.01 mM ALA-heptyl ester or to 1 mM ALA for 4 h. The decay of porphyrin fluorescence during light exposure can be fitted with a sum of two exponential decays. The photobleaching rates are similar for both drugs. The sensitivity of WiDr cells to PDT with 0.01 mM ALA-heptyl ester or 1 mM ALA for 4 h was similar. Under conditions in which the same level of PpIX is produced from ALA-heptyl ester and ALA, the intracellular localization of PpIX, the photoinactivation of cells and PpIX photobleaching rate were the same for both drugs, although a 100 times lower concentration of ALA-heptyl ester was used in comparison with ALA. ALA and ALA-heptyl ester may have different mechanisms of uptake into the cells. In normal mouse skin ALA-heptyl ester appeared to induce similar amounts of PpIX (topical application) or less (systemic application) than ALA did.

OC341

Using iron chelating agents to enhance PPIX-induced photodynamic therapy

Alison Curnow, *Andrew Pye, Sandra Campbell*

Cornwall Dermatology Research, Peninsula Medical School, Truro, Cornwall, United Kingdom

Topical protoporphyrin IX (PPIX) induced photodynamic therapy (PDT) of basal cell carcinoma (BCC) produces good clinical outcomes with excellent cosmesis as long as the disease remains superficial. Efficacy for nodular BCC (nBCC) however appears inferior to standard treatment unless repeat treatments are performed. The enhancement of this treatment modality is therefore being investigated using iron chelating agents to temporarily increase PPIX accumulation above the levels normally obtained using aminolaevulinic acid (ALA) or the methyl ester of ALA (MAL) alone.

PPIX fluorescence and cell kill was quantified in cultured human cells of dermatological origin incubated with ALA or MAL +/- the novel iron chelator, CP94 or the established iron chelator, desferrioxamine (DFO). Additionally the effect of CP94 on PPIX accumulation and PDT damage following the topical application of ALA +/- CP94 in normal rat skin was considered before conducting a small dose-escalation, safety study of the effect of CP94 in patients receiving PPIX-induced PDT treatment for biopsy proven nBCC.

Experimentally, CP94 has been found to produce greater PPIX fluorescence when administered with ALA or MAL than either congener can produce alone. CP94 has also been found to be superior to DFO in the enhancement of PPIX fluorescence. Increased PPIX fluorescence was associated with increased PDT damage both *in vitro* and *in vivo*. Clinically, the first investigation of this simple pharmacological treatment modification has demonstrated that CP94 can be used safely when treating nBCC with ALA or MAL-PDT. A significant trend of improvement in histological clearance has also been observed with increasing doses of CP94.

It is therefore concluded that the concomitant use of the iron chelating agent, CP94, during PPIX-induced photodynamic therapy is a promising technique of enhancement that warrants further investigation in a variety of clinical PPIX-PDT applications.

OC342

Photodynamic-mediated proteasome paralysis reinforced by non toxic drug may force tumour cells to apoptosis

Giuseppe Palumbo, *Angela Chiaviello*

Dipartimento di Biologia e Patologia Cellulare e Molecolare L. Califano IEOS/CNR, Naples, Italy

Irradiation of cells engulfed with a photoactivable dye may induce death or let the cell to survive, depending upon the amount of reactive species produced (singlet oxygen, radicals), the site of their generation, the state and nature of the cell. All these factors, except the nature of cells, may depend on many factors including the non-symmetrical and uneven penetration of the dye within the cell and the amount of light that penetrates the tissues and reaches the diseased cell. A neoplastic cell that survives is very dangerous because not only may restart the tumour growth, but may have acquired particular resistance to further treatments. So, to improve the efficacy of PDT, it is desirable to extend the deadly action of the photodynamic therapy by adding further treatment. Such possibility has been in part successfully exploited in vitro, when PDtr (at low dose) was coupled with administration of low doses of an antineoplastic drug whose action is directed on specific phase of the cell cycle. Recent observations made in our laboratory, have suggested a new way to study the molecular mechanisms underlying the cell response to PDtr with Photofrin at low doses of light (0,54 J/cm^{-2}) using a different approach. Indeed we have observed that in the latter conditions, PDtr determines a block of the proteasomal activity in that, while ubiquinated proteins were accumulating, the half-life of proteins like Ikb, p27 and Bcl-2, whose degradation is notoriously under proteasome control, was significantly enhanced. This block, however was transient as the proteasome activity was recovered within ~ 3 hours. Such paralysis is certainly due to oxidative stress since it could be prevented by antioxidant or prolonged by repeating the photodynamic treatment within two hours from the first irradiation. Since the prolongation of proteasome inactivity beyond reasonable times induces cell death by apoptosis, our current effort is to find and exploit suitable conditions in which the proteasome activity could be lengthily stopped without using classical toxic proteasome poisons.

OC343

ALA-PDT and Wound Healing

Stefania Motta[1], *Antonella Ferrario*[2], *Marcello Monti*[1]

[1]*Università degli Studi di Milano and Istituto Clinico Humanitas, Milano, Italy;* [2]*UO Laboratorio Analisi, Istituto Clinico Humanitas, Milano, Italy*

Wound healing is the process of restoring integrity to traumatised tissue, and involves the biological principles of tissue response to injury with all of its associated inflammatory, immune, biochemical, cell biology and signal transduction mechanisms. Photodynamic therapy with 5-aminolevulinic acid (ALA-PDT) is widely used in dermatology to destroy malignant skin tumours and induce remission in some inflammatory disorders, and some recently published data indicate that skin venous ulcers can also be targeted. Our Department has a specific unit dedicated to healing skin ulcers, in which we have been using multisession ALA-PDT for three years to treat ulcers of all pathogenetic causes. In order to establish the role of ALA-PDT in wound healing, we experimentally evaluated the efficiency of red light vs red light+ALA, the antibacterial activity of ALA-PDT, and the activity of ALA-PDT vs a standard dressing in treating 13 cases of chronic venous ulcers, four of autoimmune ulcers, three of hypertensive arterial ulcers, and two cases of ulcerated radiodermitis. All of the selected patients had multiple and/or large ulcers (area >20cm^2). After obtaining the patients' formal informed consent, multiple ulcers were divided into groups and treated with:1) our standard dressing alone; 2) our standard dressing and weekly ALA-PDT; and 3) our standard dressing and weekly red light exposure. The ALA-PDT treatment consisted of the application of 10% ALA in polyethylenglycol (PEG) ointment in occlusion for 24 hours,

followed by exposure to diode red light at 630 nm, irradiance 160mW at 50 mm, for eight minutes delivering 75 J/cm^2. Microbiological samples were collected from all of the ulcers for the primary isolation of gram-negative bacilli, gram-positive cocci and mycetes before the application of ALA or dressing, after 24 hours' ALA or dressing occlusion, and after exposure to red light.
On the basis of our 3-year experience, we can say that multisession ALA-PDT heals all of the treated ulcers, and in half the time required by the standard dressing in the same patient; red light exposure alone does not promote wound healing; and ALA-PDT does not have any antibacterial activity. These in vivo results demonstrate the favourable activity of ALA-PDT in wound healing, although the underlying biological mechanisms are still unclear.

OC344

Plea for the identification and development of new sensitizers

Thierry Patrice, David Olivier, Samuel Douillard
Cancer Photobiology, HGRL, Nantes, France

Although PDT had been approved for several clinical applications it appears to slow develop something in strong contrast with the clinical efficacy. PDT patents are very old something contradictory to companies' strategy requesting one new compound with a satisfying ROI every 5 years. Research investments are not increasing linearly but follow a precise strategy. There is a kind of balance between the need for a valid pipeline and the need for a fast access to market. One could propose the following algorithm for PDT drug identification: clinicians analyse the market and determine in cooperation with chemists what could be a good source of sensitizer, finalize a strategy. Candidates are then screened and developed internally up to the in vivo assays (included) then patented. Licensing to a company is achieved at this step before GMP manufacture upscaling and clinical assays starting. As mechanistics widely depends on chemical structure identification of new sensitizer identification is the essential key for tomorrow's PDT: without new drugs no patents then no investments then no research then no patients then no money for PDT and the ultimate no promotion or meetings!

OC345

Photochemical internalization (PCI) of the recombinant melanoma-targeting immunotoxin scFvMEL/rGel causes rapid tumor regression *in vivo*

Pål K. Selbo[1], Lawrence H. Cheung[2], Wendy Zhang[2], Michael G. Rosenblum[2], Kristian Berg[3]
[1]Department of Radiation Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway; [2]Department of Experimental Therapeutics, Immunopharmacology and Targeted Therapy Laboratory, M. D. Anderson Cancer, Houston, Texas, USA; [3]Department of Radiation Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway

Intracellular sequestration of therapeutic agents and the subsequent degradation in endocytic vesicles is a major obstacle for several drug-based therapies of cancer. The purpose of the present study was to establish *in vivo* photochemical internalization (PCI) of a tumor-targeting immunotoxin (IT) for enhanced tumor selectivity, cytosolic delivery and anti-tumor activity. PCI is based on photodynamic therapy (PDT), a photochemical method generating reactive oxygen species (ROS) after light activation of a photosensitizer. The main PDT-induced ROS product is singlet oxygen, which can destroy a number of biomolecules including membrane lipids and proteins of endosomes and lysosomes.The anti-gp240 antibody fusion toxin scFvMEL/rGel containing the powerful ribosome-inactivating plant toxin (rGel) was tested against human non-pigmented melanoma (A-375, antigen positive) and gp-240 negative bladder carcinoma T-24 cells *in vitro*, and in a xenograft with A-375 injected s.c. PCI *in vitro* was performed by light activation of cells co-incubated with scFvMEL/rGel and the endo-lysosomal targeting photosensitizers $AlPcS_{2a}$ or $TPPS_{2a}$. Cells were chased in drug-free medium 4 h prior to light exposure. Cytotoxicity was evaluated by the MTT assay 48 h post light. Mice with 50-100 mm^3 A375 tumors got one i.p. injection of 5 mg/kg $AlPcS_{2a}$ 48 hrs and 2 mg/kg scFvMEL/rGel administered once i.v. 24 h prior to 670 nm laser light exposure. Irradiance was 100 mW/cm^2 and the total light dose given was 20 J/cm^2. Tumor growth was measured twice per week until tumors reached 1000 mm^3. PCI of scFvMEL/rGel demonstrated synergistic cytotoxic effects in A-375 cells *in vitro*, while PCI of the non-conjugated rGel was 20-fold less toxic (IC_{50}) than the IT. There were no differences in toxicity between the IT and the toxin in gp-240 negative T-24 cells. Preliminary results *in vivo* show that 1/3 of the mice (n=12) demonstrated a complete response after PCI of MEL scFv-rGel. 50% of the PCI treated animals had <800 mm^3 tumors at day 110, while no mice receiving either light exposure of tumors with IT alone (n=10) or animals that received IT treatment alone (n=10) did not acquire a CR. Light exposure of tumors with $AlPcS_{2a}$ alone (PDT) resulted in 1 CR of 10 mice (day 21). This is the first *in vivo* demonstration of PCI of a tumor cell-targeting macromolecule based agent. PCI of immunotoxins may be a potent drug delivery strategy which warrant further pre-clinical evaluation.

OC346

An *in vitro* investigation of the mechanisms of δ-aminolaevulinic-acid (ALA)-photodynamic therapy (PDT)

Yuktee Dogra, Alison Curnow, Paul Winyard
Peninsula Medical School, Exeter, Devon, UK

This study investigated the molecular mechanisms of aminolaevulinic acid induced photodynamic therapy (ALA-PDT) induced apoptosis in human cultured fibroblasts (84BR) by assessing the amount/type of cell death occurring and also the reactive oxygen species being produced.
When investigating the effect of ALA-PDT on cell death, fibroblasts were exposed separately to tumour necrosis factor α (TNFα; 25 ng/ml) and/or hydrogen peroxide (1, 10, 100 or 1000 μM) as positive controls for apoptosis and reactive oxygen species (ROS) generation respectively. Cells were assessed for type of death using annexin V binding (apoptosis), propidium iodide (necrosis) and 4'-6-diamidino-2-phenylindole (total cell count) and observed with fluorescence microscopy after 45 h. A statistically significant increase in apoptosis in the treated groups (TNF α and hydrogen peroxide) was observed when compared to the non-exposed control (Student's t-test, $p < 0.05$ and two-way ANOVA, $p < 0.01$ respectively). Fibroblasts were also exposed to 0.5 mM ALA for 6 h plus red light (630 ± 15 nm; 100 J/cm^2; Paterson Lamp, Phototherapeutics, UK) for 5 minutes and assessed for cell death as above, 16 h after irradiation. The ALA-PDT exposed cells demonstrated a statistically significant increase in apoptosis compared to the non-exposed control groups ($p < 0.01$; Student's t-test).
To investigate which ROS were being produced during ALA-PDT *in vitro*, the application of electron paramagnetic resonance spectrometry (EPR) in our model has also been developed. EPR provides a direct and unequivocal identification of specific radical species. To date, the superoxide and hydroxyl radical adducts have been trapped using the hypoxanthine/xanthine oxidase system of radical generation and work is underway to characterise which radicals are involved in the mechanism of ALA-PDT in these cellular systems. This investigation therefore sheds further light on the mechanism of ALA-PDT *in vitro* and this may enable us to improve clinical treatment in the future in applications where results are currently less than desired.

OC347

The effect of photodynamic therapy with Verteporfin on epidermal growth factor receptor signaling

Thomas Stepinac, Tayyaba Hasan
Massachusetts General Hospital, Boston, MA, USA

The Epidermal growth factor receptor (EGFR) is frequently over expressed in various types of cancers and can serve as a target for cancer therapy. Synergism between EGFR–targeted immunotherapy with Photodynamic treatment (PDT) has previously been demonstrated in our laboratory in a mouse model system of ovarian cancer. The mechanism of this synergism however, remains unclear. PDT is based on the administration of a phototoxic dye, which, upon illumination with red or near infrared light, generates singlet oxygen and other reactive oxygen species, which are cytotoxic and destroy the lesion.

We report that subcurative doses of PDT with the drug Verteporfin induce a biphasic response of EGFR: (a) EGFR gets internalized in small vesicles during the first few hours following photodynamic therapy. This internalization is thought to be due to the activation of the p38 MAP kinase and phosphorylation of EGFR on certain serine/threonine residues by p38. (b) Twenty-four to forty-eight hours post PDT, the EGFR is recycled back to the surface and cells become much more sensitive to EGF stimulation than before the treatment. This could partially explain the synergism between these two treatments and also give us an indication of the best treatment schedule when combining the cytotoxic and the cytostatic treatments.

These finding might also be relevant in combination therapies involving blocking of the EGF receptor and other cytotoxic treatments like chemo- or radiotherapy.

OC348

Factors affecting substrate specificity of photosensitizers for the multidrug-resistance pump ABCG2 found on some cancers

Janet Morgan, Ravindra K Pandey, Allan R Oseroff
Roswell Park Cancer Institute, Buffalo, NY, United States

The ATP-dependent transporter ABCG2 is a multi-drug resistant pump expressed primarily in the plasma membrane of certain types of normal and cancerous cells. It can bind and efflux a wide range of structurally different classes of natural compounds and drugs. It recently was reported[1,2] that many clinically used photosensitizers including porphyrins and chlorins are substrates for the ABCG2 pump. If ABCG2 is expressed on tumors treated by photodynamic therapy (PDT), the substrate photosensitizers will be pumped out leading to low intracellular levels, decreased phototoxicity, and an increased possibility of resistance and tumor recurrence. In particular cancer stem cells a small proportion of tumor cells postulated as tumor initiating cells are thought to express ABCG2, and if these evade phototoxicity by pumping out photosensitizers they may repopulate a treated tumor There are several options to overcome such resistance, including inhibition of the pump by tyrosine kinase inhibitors or the development and use of photosensitizers which are not ABCG2 substrates. To determine some of the structural factors that affect substrate specificity for ABCG2, we used an ABCG2 transfected cell line (HEK 293) expressing high levels of the pump and its non-expressing counterpart, combined with tyrosine kinase ABCG2 inhibitors in a simple flow cytometric assay to identify photosensitizers effluxed by the ABCG2 pump. A series of conjugates of the photosensitizers, with different groups attached at different positions on the macrocycle were then tested, to examine whether a change in specificity for the pump occurred and whether such changes depended on the position or the structure/type of the attached group. We found that many photosensitizers including porphyrins, pheophorbides and purpurinimides were substrates for ABCG2, but that various groups (such as mono, di and multmeric saccharides, biotin and integrin binding molecules) conjugated to the macrocycle at positions 8, 12, 13 and 17, but not at position 3, abrogated ABCG2 specificity. The groups attached generally appeared less important than the position of attachment. This data suggests that choosing non-substrate photosensitizers would be advantageous when treating ABCG2 expressing tumors with PDT.

[1]Robey et al, 2005 Cancer Biol & Therapy 4(2) 187-194; [2]Liu et al, 2007 Clin Cancer Res 13(8) 2463-70.

IL349

Endogenous photoprotection by melanin

Bernhard Ortel
Section of Dermatology, University of Chicago Hospitals, Chicago, Illinois, United States

The protection by melanins from the detrimental effects of solar ultraviolet (UV) radiation has two major components, the molecular and physical functions of melanins and the regulation of their expression, specifically in response to acute and chronic UV exposure. Melanins are important absorbers of UV radiation and in addition have a role as radical scavengers. However, depending on the subcellular microenvironment and the composition of melanins, photosensitization may occur. Genetic deficiencies such as albinism have first illustrated the protective function of melanins and the clinical consequences of defective protection. We are now getting closer to an understanding of the adaptive responses. The melanogenic activity of human skin after UV exposure is complex and depends on ethnic parameters. Over the past few years critical steps have been identified that connect cellular responses to UV-induced DNA damage to increased pigmentation. Alpha-MSH is derived from proopiomelanocortin (POMC) and binds to the melanocortin receptors. Recent findings implicate the role of UV damage-induced activation of p53 in the upregulation of POMC. The receiving end of this regulatory mechanism, the melanocortin 1 receptor has also been well analyzed. Melanocortin 1 receptor polymorphisms are critical in altered responsiveness to alphaMSH and to UV-induced DNA damage, and are a risk factor for melanoma. There is a relatively lower incidence of melanoma as compared to non-melanoma skin cancers. This may be partially explained by the fact that melanocytes have an alpha-MSH-inducible repair function for UV-induced DNA damage that surpasses the repair capacity of epidermal keratinocytes.

IL350

UVA and UVB effects on melanocytic nevi

Piergiacomo Calzavara-Pinton, Marina Venturini, Ausilia Manganoni
University of Brescia, Brescia, Italy

The aim of the present study was to investigate whether repeated exposures to equally effective sub-erythemogenic doses of UVA or UVB can induce changes in the dermoscopic features of acquired melanocytic nevi and whether a commercial highly protective UVA- UVB sunscreen can prevent them.

Twenty volunteers with skin type II and III were randomized to receive equally effective doses of narrow- band (NB)- UVB (emission peak 312±2 nm) or UVA1 (emission range: 340- 400 nm). Three weekly exposures were delivered for 4 weeks. Three acquired melanocytic nevi with similar size and dermoscopic features were selected in each patient. During exposures, a nevus was covered with a opaque tape, another was shielded with the sunscreen and the remaining was left unprotected. At baseline and after 12 exposures (cumulative effective dose: $3000 J_{eff}\ m^{-2}$), size and dermoscopic features (degree of pigmentation, pigment network, branched streaks, brown globules and dots) of nevi were evaluated under standardized conditions by means of a digital dermatoscope. In comparison to baseline, nevi exposed to NB-UVB showed similar statistically significant changes: increased size, increase of pigment network, overall color darkening, formation of focal branched streaks, increased number and size of brown dots and globules. The same changes were seen in nevi exposed to

UVA1. Nevi that were covered with the adhesive opaque tape and nevi shielded with the UVA-UVB remained unchanged.
Therefore paracrine and endocrine signals do not seem effective in change size and pattern of unexposed nevi, under the present experimental conditions.
In conclusion, the present investigation demonstrates that acquired melanocytic nevi show similar changes of size and dermoscopic pattern following either UVB and UVA exposures and that changes may be prevented by the application of a UVA-UVB sunscreen.

IL352
Oral photoprotection
Rik Roelandts
Photodermatology Unit, University Hospital, Leuven, Belgium
Not all patients with a photodermatosis can be protected with broad-spectrum sunscreens. Some patients are too photosensitive to be treated with sunscreens. In other patients the action spectrum is too broad or is not limited to the UV spectrum. In such cases oral photoprotection is needed.
Antihistamines are mainly used in the prevention of solar urticaria. Usually a higher dosage than the normal therapeutic one is needed in the beginning of the treatment.
Antimalarial drugs are sometimes used for patients with a polymorphic light eruption, but in general their therapeutic effect is disappointing. Antimalarial drugs are mostly used in patients with lupus erythematosus and also for porphyria cutanea tarda.
Beta-carotene is the classical treatment for patients with erythropoietic protoporphyria.
Immunosuppressive drugs are mainly used in the treatment of chronic actinic dermatitis.
Psoralens are also used as photoprotectors but in combination with UVA irradiations. This treatment is only used for severe cases of polymorphic light eruption, erythropoietic protoporphyria or chronic actinic dermatitis.
A newer approach is the use of antioxidants.

OC353
Analysis of UVA and UVB irradiations on melanocytes: evaluation of the protective capacity of a sunscreen
Hélène Hernandez-Pigeon, Marie-José Haure, Sawsane Bakioui, Françoise Belaubre, Marie Charvéron
Institut de Recherche Pierre Fabre, Toulouse, France
Human skin, unlike all other organs, is continuously and directly exposed to environmental influences. Ultraviolet (UV) radiation from the sun is among the most ubiquitous damaging environmental factors from which human skin must protect itself. Both UVA and UVB radiations are not filtered out by ozone layer of the Earth's atmosphere, and there are responsible as causative factors for various skin disorders including erythema, photoaging and photocarcinogenesis. Melanocytes play a central role in the response of skin to sunlight exposure. They are directly involved in UV-induced pigmentation as a defence mechanism. However, their alteration can lead to melanoma, a process where the role of sun overexposure is highly probable. The aim of this study was to analyze the photoprotective effect of a 30 Sun Protection Factor (SPF) sunscreen on melanocytes using biological markers related to the genotoxic or metabolism impact of UV radiation. Melanocytes were exposed to UVA or UVB irradiations in the presence or not of the 30 SPF sunscreen spread on quartz slides (2 mg/cm^2). Comet assay was used to assess UV-induced DNA breaks in nuclei of melanocytes. In this study we show that 30 SPF sunscreen completely blocks UVA and UVB - induced DNA damages. Moreover, we tested the effect of UVA or UVB irradiations on melanocytes metabolism. We have then showed that UVA or UVB irradiations induce melanocytes metabolism and that 30 SPF sunscreen modulates this increase.In conclusion, our results demonstrated the photoprotective effect of 30 SPF sunscreen on melanocytes at DNA damage level, but also at metabolism level, preserving melanocytes integrity.

OC354
UV-Filter combinations under UV-A exposure: concomitant quantification of over-all spectral stability and molecular integrity
Elisabetta Damiani[1], Lucedio Greci[2]
[1]Università Politecnica delle Marche, Istituto di Biochimica, Ancona, Italy; [2]Università Politecnica delle Marche, Dipartimento di Scienze e Tecnologie Chimiche, Ancona, Italy
Efficient UV-absorbing molecules are designed to ideally protect skin against UV-light over-exposure. However, upon UV exposure they may change spectral performance or act as photo-oxidants *via* generation of free radicals and reactive oxygen species (ROS) when either alone or in combination with others. Such ROS generated upon UV exposure by labile UV-absorbers may directly or indirectly induce oxidative damage on tissue constituents in human skin, just as UV-A radiation does. Therefore information about the photointegrity of UV-filter molecules which comprises i) stable absorbance and ii) absence of UV-induced molecular breakdown, is fundamental when developing new molecular entities and formulations for sunscreen products. In this study, the influence of UV-A exposure (275 kJ/m^2) on the spectral stability and molecular integrity of equimolar quantities of seven commonly used UV-A, UV-B and new, broad spectrum UV-AB filters incorporated into phosphatidylcholine (PC)-based liposomes alone and as mixtures, suspended in a physiological aqueous environment was examined. Spectral integrity was evaluated by recording UV-absorbance spectra of the filter molecules extracted from the liposomes before and after UV-A exposure while molecular integrity was indirectly assessed *via* quantification of UV-A induced aldehydic breakdown products of PC peroxidation. The results revealed that the spectral stability of filter molecules alone or in combination (e.g. trianilino *p*-carboxyethylhexyl triazine, EHT plus ethylhexyl *p*-methoxycinnamate, OMC) does not necessarily imply absence of radical generation and that spectral lability does not necessarily have to lead to radical generation and molecular decay (e.g. OMC). In conclusion, since the simple experimental approach here employed proved capable of discriminating between essentially photostable and photounstable UV-absorbing molecules and their respective mixtures, the system might be useful as a predictor for the occurrence of photodegradation, as well as for the potential of phototoxic injury by a single UV-absorber or by a combination of UV-absorbers.

OC355
Impact of dietary lycopene on molecular markers of photoageing in human skin
Isabel Rodriguez-Blanco[1], Muneeza Rizwan[1], Lesley Elizabeth Rhodes[1], Rachel Watson[2]
[1]Photobiology Unit Dermatological Sciences University of Manchester, Manchester, United Kingdom; [2]Dermatological Sciences University of Manchester, Manchester, United Kingdom
Lycopene is a carotenoid found in tomatoes and other red fruits and vegetables, with potential health benefits attributable to its antioxidant properties. Since photoageing is believed to be partially mediated by oxidative stress, we examined the influence of dietary lycopene on distribution of markers of dermal extracellular matrix damage associated with photoageing, following acute UV-exposure of human skin.
A randomised controlled dietary supplementation study was performed in 20 healthy females (mean age 33 yrs, range 21–47 yrs; all skin type I/II) who received either 55 g tomato paste (containing 16 mg lycopene) taken in olive oil, or olive oil alone, daily for 12 weeks. Biopsies were taken from unexposed buttock skin and at 24-hr post irradiation with 3xMED UVR (UV6 lamps

290-400 nm), both at the beginning and at the end of supplementation. Samples were snap frozen at -70ºC for immunohistochemical analysis to identify procollagen-I (pCI) and fibrillin-1. Samples were blinded and randomised, with staining assessed on a five point scale.
Type I procollagen was not significantly altered by UVR before supplementation (mean±SEM: 3.09±0.10 vs 3.28±0.15 post-UVR, p=0.29), while a small, but significant increase in pCI was seen after UVR irradiation following tomato supplement (2.77±0.19 vs 3.43±0.13, p=0.046) but not after olive oil alone (2.98±0.24 vs 3.06±0.30, p=0.78). Fibrillin-1 was significantly reduced in UVR-irradiated skin compared with non-irradiated skin at the beginning of the study (3.42±0.14 vs 3.01±0.18 post-UVR, p=0.034). This UVR-induced reduction was not observed post-supplementation in either group (tomato paste: 2.81±0.20 vs 2.76±0.19 post-UVR, p=0.8; olive oil: 3.09±0.22 vs 3.25±0.22 post-UVR, p=0.65).
The post-UVR increase in pCI, an indicator of collagen synthesis, following lycopene indicates a potential response to the UVR insult facilitated by this antioxidant. In addition, both tomato paste and olive oil appear to protect fibrillin-1 from damage by UVR. Consumption of lycopene-rich products may protect key proteins related to photoageing; further parameters are under study.
This research was supported by the British Broadcasting Corporation (BBC2).

OC356

The role of sunscreens in the prevention of skin cancer: assessing the correlation between protection against erythema and DNA photodamage

Suranahi K Buglass, *Antony R Young*
King's College London/St John's Institute of Dermatology, London, United Kingdom

A critical review of the role of sunscreens in the prevention of malignant melanoma is underway as part of an EC Network of Excellence project (GenoMel) "Genetic and environmental risk factors for melanoma: translation into behavioural change". With the continuous increase of skin cancer as a concomitant of exposure to sunlight there is a pressing need to determine both the level and type of protection that can be offered by sunscreens. The sun protection factor (SPF) as currently defined, applies only to erythema from a single UVR exposure, but must evolve to biomarkers that are relevant to skin cancer. Failure to protect against such endpoints to the same level of the SPF could, in theory, enhance skin cancer risk.
Here we present data on the efficacy of sunscreens in the prevention of cyclobutane pyrimidine dimers (CPDs) as biomarkers of [non-melanoma] skin cancer. CPDs, especially thymine dimers (T=T), are the major DNA photoproducts produced by solar UVR exposure. Additional UVR-induced molecular modifications assessed include upregulation of the *p53* gene and formation of *p53* mutations.
To examine to what extent the sunscreen SPF can be correlated with DNA damage, 14 human studies that investigated the efficacy of sunscreens and their protection against T=T were reviewed. To evaluate and interpret the data, studies were classified on the basis of the irradiation source and number of doses applied. Comparisons between the SPF and DNA protection factor (DNA-PF) were made by estimating the T=T level with and without sunscreen protection. This analysis showed no apparent correlation between SPF and DNA-PF, although in general sunscreens did decrease both T=T and p53 expression. However, most of the studies had design limitations that did not allow a reliable comparison between SPF and DNA-PF. Overall; sunscreens are a useful tool to protect against UVR-induced DNA damage but protocols to determine this need to be standardized.

OC357

UV and visible free radical action spectrum for human skin and its biological relevance

Leonhard Zastrow[1], *Louis Ferrero*[1], *Dieter Kockott*[2], *Frank Klein*[3], *Norbert Groth*[3], *Jürgen Lademann*[4]
[1]*Firm: COTY / Lancaster SAM, International R&D Center, 2 rue de la Lüjernetta, MC 98000, Monaco, Monaco;* [2]*UV-Technik, Vogelsbergstrabe 27, Hanau-Stenheim, Germany;* [3]*Privatinstitut Galenus, Volmerstrasse 7A, Berlin, Germany;* [4]*Charité-Universitätstmedizin, Berlin, Germany*

The sun is the most important source of radiations in interaction with human skin. At sea level, and due to atmospheric absorption, only 6 % of the sun radiation is UV radiation, while 52% is visible radiation and about 42 % is infrared radiation.
Until now, sun protection was only focused on UVB and most recently on UVA filtration. Visible radiation was considered as safe whatever sun intensity. This can be explained because the biological end points today used to determine the degree of protection of a sunscreen product are erythema (SPF) for UVB and persistent pigmentation (PPD) for UVA.
The search for a basic process as a general starting point for the different biological effects observed on living skin after sun exposure, has directed us to the process of the free radical formation.
The interaction of sunlight with the biological system skin is the creation of excess free radicals. These free radicals are at the beginning of a cascade of molecular biological events with potential damaging and possible beneficial effects.
The electron spin resonance spectroscopy (ESR) is the only method which can directly quantify free radical production on living tissues after their impregnation by a spin trap. Therefore the aim of this work has been to apply this technology to the determination of the action spectrum for the creation of free radicals in human skin, covering UVB, UVA and Visible radiations.
Skin biopsies samples were irradiated step by step behind different cut-off filters with identical exposure times in order to quantify the different degrees of free radical production. Spectral irradiance behind the different cut-off filters were measured using a spectral radiometer in order to calculate an action spectrum based on free radical production per wavelength and per unit dose. The action spectrum covers a wavelength range from 290 nm until 700 nm. The data were analysed in the light of known biological end points, like for example erythema and skin cancer.
The surprising result is the non negligible creation of free radicals induced by Visible light, pushing the question about new requirements in skin photoprotection.

OC358

Analysis of the properties of different substrates useful for *in vitro* sunscreen tests

D. Garoli[1], *M.G. Pelizzo*[1], *P. Nicolosi*[1], *A. Peserico*[2], *M. Alaibac*[2]
[1]*CNR-INFM LUXOR Laboratory and Department of Information Engineering, University of Padova, Padova, Italy;* [2]*Unit of Dermatology, University of Padova, Padova, Italy*

In-vitro measurements on sunscreen products are extensively used to assess reliability in terms of protection and photo-stability of sunscreens and can be adopted for routinely evaluation or optimization of new formula samples. Different materials useful for in-vitro tests have been fully characterized in term of optical transmittance and reflectance properties. Extensive photo-stability tests have been performed by exposing the substrates to different spectral wavebands. Seven sunscreens formula with different protection factor SPF have been applied on each substrate to perform total transmittance measurements; in-vitro SPF defined according with the COLIPA criteria has then calculated and the obtained results have been correlated with the values reported on labels. Our experiment reports new results that demonstrate that not

all substrates are photo-stable and recommended for photo-stability tests. Moreover, advantages and disadvantages related to the use of the different substrates have become evident by the analysis of systematic measurements.

IL359

Active and passive mitigating strategies of aquatic primary producers against excessive solar radiation

Donat-Peter Häder

Friedrich-Alexander Universität, Institut für Biologie, Erlangen, Germany

Primary producers in aquatic ecosystems – both macroalgae and phytoplankton – protect themselves from excessive solar radiation especially in the UV range by active and passive mitigating strategies. Upon exposure to radiation of high irradiances the photosynthetic apparatus of many algae down regulates the electron transport rate. This is achieved by a damage and subsequent removal of the D1 protein in the core of photosystem II, which anchors the active photosynthetic pigment P680, a chlorophyll *a* dimer. In dim light this protein is re-synthesized rapidly and the photosynthetic capacity is restored. Another option against photobiological stress is the usage of specific carotenoids in the vicinity of the photosynthetic reaction centers.

In order to passively diminish the impact of solar UV radiation many macroalgae and phytoplankton produce UV-absorbing substances. Many of these substances belong to the group of mycosporine-like amino acids of which about 20 different forms are known and found in primary aquatic producers. Recently we could identify the stereostructure of porphyra-334 by an experimental and calculational NMR-investigation. There is evidence that this pigment is an efficient proton sponge. This MAA was subjected to extensive ^{1}H- and ^{13}C-NMR analysis as well as to density functional theory (DFT) calculations. All ^{1}H- and ^{13}C-NMR signals could be assigned, as well as the resonances of prochiral proton pairs, achieved by 500 MHz standard COSY, HMQC and HMBC spectra, and also by one-dimensional (DPFGSE-NOE) and two-dimensional (NOESY) spectra. Diffusion measurements (DOSY) confirm that the molecule is monomeric in D_2O.

IL360

Sensitivity to solar radiation of summer phytoplankton assemblages from mid-latitudes of Patagonia

E. Walter Helbling[1,2], M. Alejandra Marcoval[1], Paul P. Janknegt[3], Marco de Graaff[3], Ronald J. W. Visser[3], Anita G. J. Buma[3], Virginia E. Villafañe[1]

[1]Estación de Fotobiología Playa Unión, Playa Unión, Rawson, Chubut, Argentina; [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Playa Union, Rawson, Argentina; [3]Department of Marine Biology, Centre for Ecological and Evolutionary Studies, University of Groningen, Haren, The Netherlands

At mid-latitudes, along the Patagonian coast, summer marine phytoplankton assemblages are normally exposed to relatively high levels of solar radiation, especially to UVR (280-400 nm), due to a combination of low solar zenith angles, high heliophany and thermal stratification of the water column. Under this particular radiation climate, results from short term experiments (3-6 hs) indicated that these summer phytoplankton assemblages, mainly dominated by small monads and flagellates, are comparatively more resistant to UVR exposure - in terms of photosynthetic inhibition - than those from winter (dominated by large diatoms). Intensive studies carried out during summer further corroborated that UVR-induced photoinhibition is clearly dependant on species composition and size structure of the community. This size-dependence of UVR-induced damage was also observed in some Patagonian sites where high CPDs (cyclobutane pyrimidine dimers) levels were found in picoplankton but not in microplankton. We also evaluated the interactive effects of solar radiation and nutrient addition during long term experiments (1-2 weeks). Nutrient addition resulted in an overall enhancement of growth rates as well as a decrease of photosynthetic inhibition. In these experiments, a shift in the dominance of species was also noticed, suggesting a selection towards more tolerant / less sensitive ones. In fact, at the beginning of experiments, phytoplankton communities were generally dominated by monads and flagellates but by the end, diatoms comprised the bulk of biomass, with only one to four taxa dominating. The effects of solar radiation and nutrient addition were also assessed in diverse photosynthetic parameters of native species exposed to summer radiation conditions. It was seen that dinoflagellates were less inhibited by solar UVR than diatoms; furthermore, dinoflagellates synthesized great amounts of UV-absorbing compounds, providing some protection to cells. Overall, we conclude that, in summer phytoplankton assemblages of Patagonia, the responses to UVR depend on several factors but mostly on the taxonomic composition and size structure and nutrients availability.

IL361

Ultraviolet radiation tolerance and photoenzymatic repair across aquatic taxa from bacteria to fish: implications for climate change

Craig E Williamson

Miami University, Oxford, Ohio, United States of America

Climate change and other natural and anthropogenic environmental changes are altering the UV transparency of many lakes and rivers worldwide. The size, mobility, and trophic position of an organism may influence the extent to which it can utilize different defenses against changes in potentially damaging UV radiation. Which defenses an organism uses may in turn influence interactions with other components of an ecosystem. Here we use a specialized phototron that separates out photorepair wavelengths from shorter more damaging UV-B wavelengths to examine overall UV tolerance as well as the relative contribution of photoenzymatic repair (PER) to overall UV tolerance in organisms ranging from bacteria and phytoplankton to zooplankton and larval fish. UV tolerance varied greatly among species with LE50 values ranging almost continuously from less than 20 to over 350 KJ m-2. In most taxa the overall level of UV tolerance was less than the equivalent of a single day of exposure during summer solstice, suggesting that either acclimation or a daytime refuge in deeper or shaded waters is necessary to avoid high levels of mortality even under current UV exposure conditions. PER was widespread across taxa. The percentage contribution of PER to overall UV tolerance was highly variable among species, ranging from less than 10% in two benthic invertebrates to over 90% in a bacterium and a protozoan. Neither UV tolerance nor the prevalence of PER showed any consistent relationship to organism body size across a size range of 1 to greater than 10,000 micrometers. These data suggest that as climate change and other natural and anthropogenic environmental changes alter UV transparency of inland waters, the response of organisms is likely to be both highly variable and species specific rather than any particular taxonomic or ecological group showing greater intrinsic sensitivity to UV damage.

IL362

Ultraviolet radiation effects on phytoplankton productivity in a changing global climate

Patrick J. Neale[1], Cristina Sobrino[2]

[1]Smithsonian Environmental Research Center, Edgewater, MD, United States; [2]Smithsonian Environmental Research Center and University of Vigo, Vigo, Pontevedra, Spain

The strong effects of ultraviolet (UV) radiation on photosynthesis by phytoplankton will probably be modulated by several interactions with future climate change. Spatial and temporal

patterns of UV exposure will be affected by changes in patterns of cloudiness and atmospheric aerosols. Despite controls on chlorofluorocarbons, ozone depletion continues to enhance incident UV-B, particularly around the poles, due to cooling of the stratosphere. Changes in both water temperature and wind patterns will affect the strength of stratification and rates of vertical mixing of the water column. Vertical mixing modulates the light climate of phytoplankton by determining the times scales of exposure to UV and photosynthetically available radiation (PAR) This can change the overall impact of UV on water column productivity depending on rates of UV inhibition, recovery and acclimation. Finally, at the organism level, sensitivity to UV responds to multiple environmental factors that will be influenced by climate change, including overall light availability, temperature and CO_2. Some insights into the importance of these interactions has been obtained by measuring changes in the spectral sensitivity to UV inhibition (biological weighting functions, BWFs) in response to the manipulation of UV and PAR exposure, temperature and CO_2 in both laboratory cultures and field assemblages. The BWFs are then used in the context of an appropriate photosynthesis-irradiance (P-E) model and water column conditions to evaluate the possible impact of UV on water-column productivity under different combinations of environmental factors.

IL363

Plant responses to UV radiation: from signalling pathways to sustainable production

Nigel D. Paul[1], *Gareth Jenkins*[2], *Jason J. Wargent*[1]

[1]*Department of Biological Sciences, Lancaster University, Lancaster, Lancashire, UK;* [2]*Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK*

Plant responses to ultraviolet (UV) radiation are numerous, resulting in rapid and permanent alteration to numerous aspects of plant form, physiology and biochemistry. Such responses to UV radiation, and UV-B in particular, have been previously studied largely due to concerns over ozone depletion, but a recent refocus towards the effects of environmentally relevant UV-B doses has provided an opportunity to investigate the mechanistic basis for several well defined plant responses. We have characterised some of these underlying mechanisms using a combination of crop species and *Arabidopsis* mutants, in an effort to build up a model of whole-plant response. The role of UV in regulating leaf growth has been investigated using *Lactuca sativa* as a model species. We have shown in this species that reduced leaf area can be attributed to a series of changes in leaf expansion rate, leaf biophysical properties, cell growth and cell wall peroxidase content. Changes in both growth and pigmentation at the whole-plant scale are being related to regulatory pathways that have been defined previously largely in terms of gene expression. For example, we are using Arabidopsis mutants deficient in the UVR8 system to demonstrate the key role of this regulatory system, which regulation of pigment synthesis genes in response to UV-B, in determining growth and pigment formation across a range of UV-B doses and spectral distributions. The implications of UV responses in the wider context of climate change are also being considered. Do changes resulting from UV exposure influence plant responses to environmental stress, and can manipulation of the UV environment of crops contribute to approaches to sustainable agriculture in the face of changing climate?

OC364

Fitting action spectra: ambient levels of UV-A and UV-B radiation affect phenolic compounds in birch leaves

Titta Katariina Kotilainen[1], *Tuulia Venäläinen*[1], *Riitta Tegelberg*[2], *Riitta Julkunen-Tiitto*[3], *Pedro Jose Aphalo*[2]

[1]*Dept. Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland;* [2]*Dept. Biological and Environmental Science, University of Helsinki, Helsinki, Finland;* [3]*Natural Product Research Laboratory, Dept. Biology, University of Joensuu, Joensuu, Finland*

We studied the accumulation of individual UV-absorbing phenolics, chlorophyll concentrations and growth attributes in response to solar UV-A and UV-B radiation in silver birch (*Betula pendula*) seedlings. Our experimental setup was based on the use of plastic films excluding different parts of the natural UV spectrum. The experimental setup consisted in six treatments in five blocks: 1) UV-B 100%, UV-A 100% (near ambient control), 2) UV-B 0%, UV-A 100%, 3) UV-B 0%, UV-A 0%, 4) UV-B 50%, UV-A 100%, 5) UV-B 50%, UV-A 50%, 6) UV-B 0%, UV-A 50%. The experiment was done outdoors with seedlings growing in pots. The films were attached to wooden frames on the top of the seedlings. Measurements on seedling height growth and leaf, petiole and stem dry weight and chlorophyll concentrations showed no significant effect of UV-exclusion. Analyses by HPLC showed that both ambient UV-A and UV-B radiation enhanced the accumulation of several phenolic compounds in birch leaves. UV-B radiation affected more compounds than UV-A radiation. The attenuation of UV radiation always decreased UV-absorbing metabolites in comparison to the concentrations found under ambient control. In agreement with earlier indoor photophysiological studies we now show that under outdoors conditions, both UV-A and UV-B are important regulators of the accumulation of individual phenolic metabolites. This indicates that at least for some UV-absorbing metabolites the action spectrum fitting the data needs to include action also for UV-A radiation.

OC365

How the DNA composition and the physiological state of marine bacteria may modify their DNA damages and their sensitivity to UV radiation

Sabine Matallana Surget[1,2], *Fabien Joux*[1], *Jarah Meador*[1,3], *Thierry Douki*[3], *Rick Cavicchioli*[2]

[1]*Laboratoire d'Océanographie Biologique de Banyuls, CNRS URM7621, Université Paris VI, Avenue Fontaulé, BP44 F-66651 Banyuls-sur-mer Cedex, France;* [2]*School of Biotechnology and Biomolecular Sciences, The University of New South Wales, Sydney, 2052 NSW, Australia;* [3]*Laboratoire des Lésions des Acides Nucléiques, DRFMC/SCIB UMR E-3 CEA/UJF, Grenoble, France*

Solar UV radiation reaching the Earth's surface (290-400 nm), and especially the most energetic UVB portion (280-320 nm), is widely recognized as damaging for organisms living at the surface of aquatic ecosystems. UVB radiation generates two major photoproducts, namely cyclobutane dimers (CPDs) and (6-4) photoproducts (6-4PPs). Bacteria are especially sensitive to these damages because they have a haploid genome with little or no functional redundancy. However little is known concerning the induction of DNA damage in bacteria differing by their genome composition or their physiological states. In this study we compared the changes in viability and DNA damages for two marine bacteria differing by their GC content (*Sphingopyxis alaskensis* RB2256 and *Vibrio angustum* S14, respectively facultative oligotrophic and copiotrophic bacteria). Moreover, the effects of the growth temperature and the growth phase on the sensitivity to UVB were also studied in the case of *S. alaskensis*. The content in UV-induced DNA damage was determined by liquid chromatography associated to tandem mass spectrometry to provide information of all possible bipyrimidine photoproducts. We observed that the proportion of the different photoproducts varied drastically according to the GC content of bacteria for both cellular

and isolated DNA. Moreover, while *S. alaskensis* provided an exponential increase of the photoproducts amount during all the UVB exposure, *V. angustum* presented a stationary in its DNA damages. In a surprising way, *V. angustum* could remove even during the UVB exposure some of its DNA lesions (TT and TC CPD). Moreover, concerning the DNA repair, photoreactivation was efficient for both bacteria while dark repair was more efficient for *V. angustum*. Qualitatively, 6-4 PPs were removed more efficiently than CPDs for both bacteria. The temperatures studied (12 and 24°C) and the growth phases (stationary and late stationary phases) did not affect the composition of DNA photoproducts induction and the sensitivity of *S. alaskensis* to UVB. Yields of DNA photoproducts were also similar between the different conditions except for the late stationary phase at 24°C for which higher quantity of damages where measured. These observations provide new insights in the photobiological impact of UVB on aquatic bacteria, especially regarding the consequences of the GC content on the mutational properties of UVB-induced photoproducts.

OC366

The effects of UV radiation on multitrophic responses and their implications for sustainable agroecosystem management

Jason J. Wargent, *Nigel D. Paul*

Department of Biological Sciences, Lancaster University, Lancaster, UK

One vital aspect of understanding ecological responses to climate change is how complex environmental changes impact on biological interactions delivering what have been termed “ecosystem services”. These include a range of multitrophic interactions where we have relatively limited understanding of the effects of UV radiation. Here we examined the effects of elevated UV in two multitrophic systems: (1) Plant-insect-insect interactions with a typical model system of host plant, herbivore and parasitoid. Female adult *Plutella xylostella* oviposited preferentially on *Brassica oleracea* plants grown in a zero ultraviolet-B (UV-B) environment compared to plants under elevated UV-B, and *P. xylostella* larvae also fed preferentially on non UV-B exposed leaf material compared to UV-B supplemented tissues. In contrast, female parasitoids (*Cotesia plutellae)* were preferentially attracted to UV-B exposed plants grazed by *P. xylostella*. These UV mediated responses are discussed in the context of interactions between pathways of plant response to both UV-B and herbivory. (2) Plant-microbe-microbe systems with the assessment of UV sensitivity in the fungal plant pathogen *Botrytis cinerea* and its antagonistic *Trichoderma harzianum*, which is an established biocontrol agent for *B. cinerea*. Exposure to increased UV inhibited not only *B. cinerea* conidiospore germination, but also that of *T. harzianum*, which showed markedly increased sensitivity to UV-A wavelengths compared to *B. cinerea*. Better understanding of such UV responses can contribute both to understanding responses to climate change and developing mitigation strategies as they relate to agriculture, particularly making optimum use of sustainable approaches to pest and disease control.

OC367

Singlet oxygen affects activity, cells size and species composition in a dystrophic humic acid lake

Stefanie Glaeser[1], *Bork Berghoff*[1], *Hans-Peter Grossart*[2], *Jens Glaeser*[1]

[1]*Justus-Liebig-Universitaet, Institut fuer Mikrobiologie und Molekularbiologie, Giessen, Germany;* [2]*Leibniz-Institut für Gewaesseroekologie und Binnenfischerei, Stechlin, Germany*

The photolysis of dissolved organic matter (DOM) generates small carbon substrates and reactive oxygen species (ROS) in natural waters exposed to light and oxygen. Therefore, contrasting inhibitory and stimulating effects may affect the activity and species composition in epilimnic bacterial communities. Ozone depletion and subsequently increased UV radiation changes the rate of DOM-photolysis. Consequently ROS may affect on bacterioplankton communities to a larger extent. We investigated effects of increased and decreased levels of oxidative stress by artificial singlet oxygen ($^1O_2$) formation and scavenging in a humic acid rich lake. The addition of $^1O_2$ quencher furfuryl-alcohol to water samples increased the fraction of actively respiring bacteria by 10 fold during *in situ* incubations (5h, daylight) and by 5 fold during incubations in the laboratory (3 days, artificial daylight). Surprisingly the artificial generation of $^1O_2$ using Rose Bengal did not affect the fraction of respiring bacteria but increased the fraction of bacteria smaller than 0.5 µm in diameter. Changes in the oxidative stress levels affected bacterial community structures and activity patterns as shown by PCR and RT-PCR based fingerprinting using Denaturating gradient gel electrophoresis (DGGE). In this approach fragments of the 16S rRNA gene or of 16S rRNA were amplified from particle-associated (>8.0 µm) and free-lifing (0.2-8.0 µm) bacterioplankton with universal bacterial primers and primers targeting different bacterial groups as *Actinobacteria*, *Beta*- or *Alphaproteobacteria*. Thereby species either resistant or sensitive to singlet oxygen were identified. In parallel, stress resistant and stress sensitive species were isolated from the respective treatments using MPN dilution series. Subsequent screening with DGGE identified environmentally relevant bacteria among the obtained isolates. Respective strains were used for inhibition zone assays to investigate in further detail their response to $^1O_2$ and other ROS.

PL401

Impacts of UV-B radiation on biotic and abiotic systems in relation to global climate change

Janet F. Bornman

International Global Climate Institute, IGCI, University of Waikato, Hamilton, New Zealand

Research on the effects of UV-B (280-315 nm) radiation has in recent years increasingly focussed on interaction processes from the molecular level all the way to the macro dimension where different environmental factors can be seen to elicit a complex array of responses at the whole organism level. The molecular mechanisms underlying plant and animal response to simultaneous abiotic and biotic stresses, rather than to single stress events, have only fairly recently been investigated. This approach is important, since response to multiple stresses is often unique and not immediately indicative of each stress applied alone.

Implications of ozone depletion, consequent elevated UV-B radiation and interactions of climate change factors are posing many unknowns for life on earth. The most significant climate change factors include increasing greenhouse gas emissions, rising temperatures in mid-latitudes, frequent flooding and drought events. These occurrences result in a wide range of consequences for plants and animals, among them shifts in seasons, spreading of vector-borne diseases into new areas, changes in species composition and abundance, a plethora of indirect effects, including impacts on ecosystem processes below soil surfaces. Added to this disturbance, modern agricultural practices and multi-level pollution are poised to further unbalance life on earth unless concerted action is taken.

The key findings for the environmental effects of an enhanced UV-B radiation as a consequence of a depleted ozone layer in conjunction with other climate change factors include, with respect to human health, damage to the eyes, skin cancers and suppression of the immune system. This suppression appears linked to the increasing occurrence of skin cancers. The biological availability and toxicity of metals and alterations in carbon and nutrient cycling in plant and aquatic ecosystems are enhanced by increasing UV-B radiation. A stressed biological system may show resilience in some situations, but become more susceptible, e.g., to disease in others. In the former case the phenomenon of cross-tolerance

reflects some of the synergistic relationships among different stresses that may lead to plant hardening and acclimation. Regulatory processes and plant acclimation to the altered environmental conditions have recently been investigated through studies on signal transduction pathways and changes in gene expression. Apparent key signaling elements in these pathways, reactive oxygen species (ROS), lead, through triggering of defence mechanisms, to regulation and acclimation and are themselves usually increased by climate change factors such as enhanced levels of UV radiation, temperature extremes, drought, ozone and disease. Other signaling components such as UV-B-specific regulatory proteins and defense-related hormones may operate in overlapping pathways in response to multiples stresses which in turn result in shared gene expression patterns and convergent response.
One may ask, is climate change simply due to natural solar variability or are we seeing unprecedented accelerated change and complex interaction events that are instead due to human activities?

IL403
Mechanisms of UVA- and sunlight-induced mutations

Gerd P. Pfeifer, Sang-in Kim, Ahmad Besaratinia
Division of Biology, Beckman Research Institute, City of Hope, Duarte, CA, United States

The different ultraviolet (UV) wavelength components, UVA (320-400 nm), UVB (280-320 nm), and UVC (200-280 nm), have distinct mutagenic properties. A hallmark of UVB and sunlight mutagenesis is the high frequency of transition mutations at dipyrimidine sequences containing cytosine and particularly 5-methylcytosine. On the other hand, UVA induces oxidative DNA damage and G to T transversions. We investigated the kinetics of repair for UVA- and UVB-induced lesions in relation to mutagenicity in transgenic mouse embryonic fibroblasts irradiated with equilethal doses of UVA and UVB in comparison to simulated sunlight (SSL). Cleavage assays with specific DNA repair enzymes revealed that both UVB- and SSL-irradiation, but not UVA-irradiation, induced CPDs and (6-4)PPs, in the genome overall of irradiated cells. Whereas the induced (6-4)PPs were repaired within 6 h after both UVB- and SSL-irradiation, the CPDs remained persistent for at least 24 h. Also, UVA- and SSL-irradiation alike formed significant levels of oxidized purines in the genome overall of irradiated cells; however, the induced lesions were completely repaired within 30 min post-irradiation. Footprinting of DNA damage/repair at sequences along the *cII* transgene confirmed these findings. Determination of *cII* mutant frequency showed that UVA-irradiation was weakly but significantly mutagenic, whereas SSL- and UVB-irradiation were extremely mutagenic. The induced mutation spectra by UVB- and SSL-irradiation were both characterized by a significant increase in the relative frequency of C to T transitions at dipyrimidines. This type of mutations accounted for 85% and 80% of the observed mutagenicity of UVB- and SSL-irradiation, respectively. The G to T transversions induced by UVA-irradiation, however, contributed only to 3.3% of the mutation load imposed by SSL-irradiation. We conclude that the prevailing role of UVB over UVA in solar mutagenesis is due to different kinetics of repair for lesions induced by the respective irradiation.

IL404
UVA-induced response in eucaryotic cells

Evelyne Sage[1], Violetta Smirnova[1], Anne Reynaud-Angelin[1], Pierre-Marie Girard[1], Stefania Francesconi[1], Delphine Dardalhon[1], Ludovic Tessier[1], Stanislav Kozmin[1], Thierry Douki[2]
[1]CNRS UMR2027, Institut Curie, Orsay, France; [2]2DRFMC/SCIB UMR-E 3 CEA-UJF; CEA, Grenoble, France

Ultraviolet A (320-400 nm) radiation constitutes more than 90% of the environmentally relevant solar UV radiation and it has been proposed to play a role in skin cancer and ageing. Due to the popularity of the high-intensity UVA tanning equipment and prolonged periods of sunbathing, the potential deleterious effect of UVA has emerged as a source of concern for public health. Although generally accepted, the impact of DNA damage on the cytotoxic, mutagenic and carcinogenic effect of UVA radiation remains unclear. We undertook to investigate the toxicity and DNA damage induction by UVA radiation in different eucaryotic cells and observed that the cellular sensitivity and the ratio of cyclobutane pyrimidine dimers to 8oxoguanine vary drastically, depending on the conditions of irradiation. Since DNA damage is well known to induce activation of checkpoint pathways that delay cell cycle progression, we explored effects of UVA on cell cycle in yeast *Schizosaccharomyces pombe* and in human cultured cells. In both organisms, cell cycle checkpoints and oxidative stress response pathway are activated and a dose dependent S-phase slowdown is observed. Anyhow, the delay in DNA replication does not depend on activation of checkpoints and stress response pathways. Data from the study in fission yeast strongly suggest that UVA impinges on DNA replication by inducing collapse of replication forks. This leads to formation of double strands breaks (DSBs) that are a major challenge to genome integrity. These damages result in a checkpoint dependent cell cycle arrest at the following G2 phase. Altogether, our data point out a specific response of eucaryotic cells to UVA radiation and emphasize the potential harm of UVA radiation, particularly in replicating cells, a quite relevant finding considering that UVA penetrates the proliferative basal layers of epidermis.

IL405
Genotoxic effects of UVA radiation on human cells and skin: photosensitized reactions

Jean Cadet, Stephane Mouret, Jean-Luc Ravanat, Thierry Douki
CEA/Grenoble, Grenoble cedex 9, Isère, France

Major progress has been achieved during the last decade in a better understanding of the molecular effects of UVA radiation, the most important UV component of solar light and for a while underestimated in term of biological impact, on cellular DNA. It is now assumed on the basis of experimental data so far available from several research groups that genotoxic effects of UVA photons are accounted for by two main photosensitization mechanisms according to an oxygen dependent and independent pathway respectively. The role of oxidative processes that has been inferred in particular from the observed induction of heme oxygenase and the measurement of 8-oxo-7,8-dihydroguanine (8-oxoGua) in DNA has received confirmation. However, as discussed later the oxygen dependent photochemical process appears to be of lower intensity that supposed initially. It is reasonable to assume that $^1O_2$ that is produced by a likely type II photosensitization mechanism is at the origin of the formation of 8-oxoGua in the DNA human keratinocytes, fibroblasts and skin explants. It may be added that $^1O_2$ is unable to induce the formation of DNA strand breaks in contrast to earliest claims. Furthermore the relatively minor formation of strand breaks and endonuclease III-sensitive sites which are indicative of oxidized pyrimidine lesions are suggestive of the implication of OH radical as the result of the occurrence of Fenton type chemistry. The main DNA degradation pathway is however photodimerization of adjacent pyrimidine bases which gives rise to the predominant formation of cyclobutane dimers at TT sites and to a lesser extent TC sequences as inferred from accurate and HPLC-tandem mass spectrometry measurements in the electrospray ionization mode. The photoproduct distribution together with the lack of detectable induction of pyrimidine (6-4) pyrimidone photoadducts whose formation involves a single excited state is strongly suggestive of the occurrence of a triplet energy transfer mechanism although the putative endogenous photosensitizer(s) implicated remain to be identified. The ratio 8-oxoGua/cyclobutadipyrimidines that varies in biological samples in

the following decreasing order: fibroblasts > keratinocytes > skin is indicative of differences in the photosensitizer contents of the cells. As a final major remark, the about 15-fold higher relative efficiency for UVA radiation to degrade DNA in skin than UVB photons when compared with those on cells is in agreement with a better penetration of UVA light in the dermis.

IL406

UVA stress to human skin cells and the maintenance of heme and iron homeostasis

Rex Michael Tyrrell[1], *Chintan Raval*[2], *Gavin Edwards*[2], *Julia Zhong*[2]

[1]*Department of Pharmacy and Pharmacology, University of Bath, Bath, United Kingdom;* [2]*University of Bath, Bath, United Kingdom*

Organisms ranging from the simplest procaryotes have evolved stress responses largely to cope with abrupt changes in the external environment. Much has been learnt about regulation of gene expression from understanding how key stress genes are maintained in a quiescent state under stress free conditions but remain poised to respond immediately to change. A knowledge of these pathways is also crucial to understanding homeostatic mechanisms. Mammalian cells respond to a large range of stresses including heat, toxic agents (e.g. heavy metals) and oxidants. A key example of the latter is the strong induction of heme oxygenase 1 which was originally observed following treatment with physiologically relevant doses of the environmental oxidant, UVA radiation. The negative regulation of this anti-inflammatory oxidant stress proteins is crucial to cellular homeostasis under stress free conditions. The clearest molecular model to emerge for regulation at the transcriptional level involves the dynamic exchange between Nrf2/MafK transcriptional activation complexes and Bach1/MafK suppressor complexes at the pair of cis-acting elements (MARE, StREs, AP-1) located in the mouse and human HO-1 promoter upstream region. Although Nrf2 has now been implicated as at least partially responsible for the activation of numerous stress and phase 2 proteins, the range of stress response which involve negative regulation by Bach1 is only now becoming understood. In the specific case of UVA radiation, we have examined the role of heme (released by UVA radiation) Nrf2, and Bach1 in HO-1 regulation. Both changes in heme status and Nrf2 are clearly involved in the up-regulation by UVA and heme and Bach1 are involved in maintaining the quiescent state. Under acute UVA stress, heme is released and Nrf2 accumulates in the nucleus while Bach1 binds to heme, loses its DNA binding and is exported from the nucleus resulting in active transcriptional up-regulation of HO-1. The removal of heme by the induced heme oxygenase and *de novo* synthesis of Bach1 leads to the develop of refractoriness to further induction by UVA and heme. Removal of heme by the novel constitutive expression of HO-2 in human keratinocytes appears to dampen the HO-1 responses in these crucial cells as shown by knock-out HO-2 expression using SiRNA and we are investigating whether this leads to corresponding changes in nuclear expression and transport of Bach1.

OC407

Tryptophan-derived UV-filter compounds covalently bound to lens proteins are photosensitizers of oxidative damage

Jasminka Mizdrak[1], *Joanne F. Jamie*[1], *Peter G. Hains*[2], *Roger J.W. Truscott*[2], *Michael J. Davies*[3]

[1]*Macquarie University, Sydney, NSW 2109, Australia;* [2]*The Save Sight Institute, Sydney, NSW 2001, Australia;* [3]*The Heart Research Institute, Sydney, NSW 2050, Australia*

The lens of the human eye is chronically exposed to light of $\lambda >$ 300 nm. This exposure has been linked to the development of age-related nuclear cataract. Light of wavelength 300 - 400 nm is predominantly absorbed by Trp residues on proteins, and free Trp derivatives such as kynurenine (Kyn), 3-hydroxykynurenine (3OHKyn) and 3-hydroxykynurenine glucoside (3OHKynG). These compounds are poor photo-sensitisers, thereby limiting photo-oxidative damage to the lens. The level of these Trp derivatives decrease with age due to deamination and binding to lens proteins. The behaviour of the protein-bound adducts is poorly understood, and it has been postulated that these species may initiate photo-oxidative damage to the lens; this hypothesis is explored in this study. Illumination of the protein adducts of the Trp metabolites gives significant concentrations of peroxides (principally H_2O_2) with this dependent on the metabolite, photolysis time and illumination wavelength, with shorter wavelengths giving higher yields. Unmodified proteins, and non-illuminated modified proteins gave low peroxide yields. Protein adducts of 3OHKyn (principally at Cys residues) gave higher peroxide levels than the Kyn and 3OHKynG adducts. Trp metabolites that do not bind to proteins, or the free species, gave little peroxide when illuminated with lens proteins. Studies using D_2O and azide gave higher and lower peroxide yields respectively, consistent with a role for $^1O_2$. The consequences of these photochemical reactions have been examined by quantifying protein-bound Tyr oxidation products (DOPA, dityrosine), and protein crosslinking. 3OHKynG-modified proteins gave elevated levels of dityrosine, but not DOPA, whereas 3OHKyn-modified protein gave the inverse. DOPA formation was independent of illumination, with this believed to arise via *o*-aminophenol autoxidation. Both reducible (disulfide) and non-reducible cross-links were detected on illuminated modified proteins. Overall these data indicate that covalent addition of Trp-derived compounds to lens proteins yields efficient photo-sensitisers. The increased concentration of these adducts in aging lenses may exacerbate UV-induced photo-damage to the lens and retina. Multiple processes appear to contribute to the enhanced level of peroxides and the protein modification detected in this system, with $^1O_2$ being a key intermediate. The interaction of UV light with protein-bound Trp metabolites may contribute significantly to an enhanced oxidative stress in older lenses, and contribute to age-related nuclear cataract.

IL408

311 nm UVB vs. PUVA: is 311 nm always best?

John L M Hawk

St John's Institute of Dermatology, London, UK

Narrowband UVB (311nm) phototherapy (NB-UVB) is steadily replacing psoralen photochemotherapy (PUVA) as the irradiation modality of choice for phototherapy-responsive skin disease worldwide. This is because of its greater convenience for patients, with no prior need to take medication or bathe in psoralen-containing water, or to wear sunglasses afterwards, and because of its apparently much reduced likelihood of inducing short or long-term adverse effects, all while having approximately equal efficacy to PUVA. However, PUVA has previously appeared very effective with few adverse effects if patients are supervised carefully in both the short and long term. We have therefore now carefully assessed the efficacy and short-term safety of the two modalities in double-blind randomised controlled trials in 93 patients with psoriasis and 56 with vitiligo. The patients were treated till clearance or a stable non-progressive state, and then followed for twelve months to assess relapse, the names of the treatment cabinets being masked and the narrowband UVB-treated patients taking placebo psoralen and wearing protective sunglasses to preserve treatment blinding. For psoriasis, PUVA was significantly more effective than NB-UVB at achieving clearance ($p=0.02$) among white-skinned patients, being effective in 84% as compared with 65% for NB-UVB, with significantly lower median treatment numbers also in the PUVA group (17 vs. 28; $p<0.001$). 49% of the PUVA as compared with 22% of the NB-UVB patients reported erythema at some stage during treatment ($p=0.004$), but six months after treatment cessation, 68% of the PUVA as compared with 35% of the NB-UVB patients were still in remission. For vitiligo, however, 64% of the NB-UVB as compared with 36% of the PUVA patients

showed greater than 50% improvement, with the colour match of the repigmented skin being excellent in 100% of the NB-UVB but only 44% of the PUVA patients ($p<0.001$). Among patients who completed 48 sessions, the improvement in affected body surface area was also greater for NB-UVB than for PUVA ($p=0.007$), while twelve months after treatment cessation, the NB-UVB superiority was maintained. It is therefore clear that PUVA can sometimes be considerably more effective than NB-UVB, and equally that the reverse may be true, depending on the condition treated. Therefore PUVA should still very much be retained as a treatment option where possible, though if space, funding or staffing constraints restrict such choice, NB-UVB is now perhaps a better single unit choice.

IL409

Topical PUVA

Herbert Hönigsmann

Medical University of Vienna, Department of Dermatology, Vienna, Austria

Topical or PUVA bath therapy was introduced in Scandinavia in the 1970s but it took several years until it attracted attention in other parts of the world. It has several advantages over oral PUVA: It provides for a uniform drug distribution over the skin surface, very low psoralen plasma levels and a quick elimination of psoralens from the skin. Bathwater delivery of psoralens circumvents gastrointestinal malaise and phototoxic hazards to the eye because there is no systemic photosensitization. Skin psoralen levels are highly reproducible and photosensitivity lasts not more than two hours. Several studies have compared bath-water delivery of 8-MOP with oral administration showing that bath-PUVA has the lowest incidence of treatment failures and overdose episodes. When comparing the results with oral 8-MOP, bath-PUVA showed equal clearing rates with lower numbers of exposures. The greater therapeutic efficacy could be due to a higher penetration of psoralens through the abnormal stratum corneum as compared with healthy skin. Based on studies in Scandinavia bath-PUVA seems to bear no relevant risk of carcinogenesis. In conclusion, bath water delivered PUVA represents a highly effective alternative treatment method to oral PUVA that is quite well accepted by the patients. The fact that lower cumulative doses of UVA are necessary may possibly reduce the risk of carcinogenesis.

IL410

UVA-1: has it kept its promise?

Adrian Tanew

Division of Special and Environmental Dermatology, Medical University of Vienna, Vienna, Austria

The term UVA1 has been coined by Thomas Fitzpatrick in 1985 and denotes longer wave UVA radiation extending from 340 – 400 nm. UVA1 phototherapy has been introduced in clinical use in the early 90s as an effective and exciting new treatment modality for acute exacerbated atopic dermatitis. Initial treatment protocols advocated high dose UVA1 exposures (single exposure doses of 130 J/cm^2) 5 times weekly over three consecutive weeks. Subsequently, a large number of clinical investigations have been conducted in atopic dermatitis and various other (mostly inflammatory) dermatoses and provided a large body of data on efficacy and tolerability as well as long-term outcome. Concurrently, the original concept of high-dose UVA1 phototherapy has been increasingly replaced by medium (50-70 J/cm^2) and low-dose (20 J/cm^2) UVA1 treatment. Nowadays, besides atopic dermatitis, urticaria pigmentosa, granuloma anulare and mycosis fungoides, localized scleroderma and graft-versus-host disease have become the most important indications for UVA1 phototherapy.

IL411

Extracorporeal photoimmunotherapy: an update

Robert Knobler

Medical University of Vienna, Department of Dermatology, Division of Special and Environmental Dermatology, Vienna, Austria

Extracorporeal Photoimmunotherapy (ECP), was developed in 1985 and has since received FDA approval for its palliative use in the treatment of CTCL Recent studies have provided important leads for the better understanding of its mechanisms of action (apoptosis, induction of regulatory T-cells, GvHD equivalent animal models, etc.) as well as documentation for potential major new indications: treatment of acute and chronic Graft versus Host disease after allogeneic bone marrow transplantation, control of rejection in solid organ transplantation (Heart, Lung, Kidney), systemic sclerosis, and other T-cell mediated diseases including Chron's disease, rheumatoid arthritis, and nephrogenic fibrosing dermopathy(NFD) and refractory atopic dermatitis.Both FDA and recent EC approval of the extracorporeally applied form of liquid psoralen (UVADEX) has further improved efficacy, reproducibility, and reliability associated with a very low side effect profile of this exciting form of cellular photoimmunotherapy. New data obtained from the results of recent prospective randomized clinical trials, particularly in the fields of hematology (acute and chronic GVHD), dermatology (systemic sclerosis, NFG), rheumatology (refractory rheumatoid arthritis), gastroenterology (refractory Chron's disease) and solid organ transplant rejection (heart and lung) have provided important information that will further help better define its role in photomedicine and inter-disciplinary medicine.

As the mechanisms of action responsible for the already observed effects get further unraveled, it is clear that the potential remains significant for further exciting contributions of this therapy in the areas of photomedicine, photodermatology, photoimmunology , oncology and medicine in general.

IL412

Molecular mechanisms of photochemotherapy: significance of PAF and 5-HT

Peter Wolf

Medical University of Graz, Department of Dermatology, Research Unit for Photodermatology, Graz, Austria

PUVA photochemotherapy has immunosuppressive and proapoptotic effects, possibly being responsible alone or in combination for its therapeutic efficacy. However, the exact molecular mechanisms, by which PUVA leads to its effects, are not well understood. Mechanistic studies in humans are difficult to undertake, we therefore have to rely largely on animal data. We have previously shown in mice by the use of platelet-activating factor (PAF) receptor antagonists that PAF is critically involved in many PUVA-induced effects (Wolf et al, AJP, 2006). Because certain PAF receptor antagonists have dual activity blockading both PAF and serotonin (5-hydroxytryptamine, 5-HT) receptor binding, we then tested the hypothesis that 5-HT might be a key mediator in PUVA-induced effects as well. The i.p. injection of mice with 5-HT_2 receptor antagonists or an anti-5-HT antiserum immediately before PUVA exposure totally abrogated systemic immune suppression (delayed-type hypersensitivity to *C. albicans*) but had no significant effect on inflammation or apoptosis. Importantly, i.p. injection of mice with 5-HT led to immune suppression in a manner similar to PUVA exposure but did not induce inflammation or apoptosis in the skin (Wolf et al, 36th Annual ESDR meeting, Paris, France, 2006). Mast cells are an important source of 5-HT and therefore we have used mast cell deficient mice to substantiate our findings on the role of 5-HT (Wolf et al, submitted, 2006). We found that mast cell deficient mice were resistant to topical PUVA-induced immune suppression compared to their wild type littermates. In contrast, PUVA-induced inflammation, as measured by macroscopic skin swelling did not differ between mast cell

deficient mice and their wild type controls. Moreover, immunohistochemical 5-HT and mast cell stainings did not reveal an upregulation of 5-HT positive cells in the skin upon PUVA exposure. These results indicate that (prestored) mast cell-derived 5-HT is a key mediator of PUVA-induced immune suppression. The fact that 5-HT is selectively responsible for PUVA-induced immune suppression but not inflammation or apoptosis opens the doors to selective dissection of PUVA's therapeutic from its harmful (i.e. carcinogenic) effects. Our current work therefore addresses the question how interference with the PAF and/or 5-HT pathway may affect the therapeutic efficacy of PUVA.

IL413

Molecular risk assessment of rhinophototherapy for the treatment of seasonal allergies

David L Mitchell[1], *Andrea Koreck*[2], *Edina Garaczi*[2], *Zsolt Bella*[3], *Lajos Kemeny*[2]

[1]Department of Carcinogenesis, The University of Texas MD Anderson Cancer Center, Smithville, Texas, USA; [2]Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary; [3]Department of Otolaryngology, Head and Neck Surgery, Szeged, Hungary

Rhinophototherapy (RPT) is an effective alternative treatment for alleviating the symptoms of severe seasonal allergies. However, the risks associated with UV induced DNA damage in nasal mucosa are not well understood. We used DNA photoproducts as biomarkers to address this issue. Radioimmunoassay was used to quantify cyclobutane pyrimidine dimer and (6-4) photoproduct induction and repair in DNA purified from two milieu, including nasal cytology samples from patients undergoing RPT and in an artificial tissues produced by MatTek Corporation. In patients, DNA damage frequencies were determined prior to and immediately after treatment and at increasing times post-treatment. We found significant levels of DNA damage immediately after treatment, consistent with the UV dose, and efficient removal of the damage within a few days after treatment in response to DNA repair mechanisms or damage dilution by cell proliferation. To better understand the molecular response of the nasal epithelium to DNA damage we conducted parallel experiments in artificial respiratory epithelium (Epi-Air) and epidermis (Epi-Derm). Induction frequencies were significantly lower in the artificial epidermis compared to the respiratory epithelium at the same dose due to shielding by the stratum corneum. However, repair rates in these two tissues were very similar and comparable to that observed in human skin. Our results suggest that the DNA damage response of respiratory epithelia is very similar to that of the human epidermis and can be used in risk assessment of RPT for photocarcinogenesis.

OC414

Erythema effectiveness of long-wave UV radiation - LASERS versus conventional radiation

Helmut Piazena[1], *Hans Meffert*[2]

[1]Klinik für Psychiatrie und Psychotherapie der Charité, Berlin, Germany; [2]Dermatologisches Zentrum Berlin, Berlin, Germany

Long-wave UV radiation is of increasing interest in the treatment of skin diseases, tanning, premature skin aging and cancer. Calculated erythema effectiveness of mostly UV-A or UV-A1 emitting devices differs up to a factor of two whether the action spectrum recommended by the CIE (1982) or the LASER supported action spectrum of Anders et al. (1995) was used. To learn about possible causes of these differences, threshold exposure times and doses predicted by calculation with both action spectra were related to erythema formation and pigmentation on the previously unadapted back skin of volunteers determined by measuring the Colorimetric Erythema Index (CEI) and the Individual Typological Angle (ITA°). The volunteers were exposed with a therapeutic UV-A1 device (CL 300.000, Photomed, Gehrden, Germany) emitting mostly UV with wavelengths between 340 nm and 400 nm and in addition by using an UV-B Erythemtester (Meffert and Dietz, 1982, equipped with a TL-12 bulb, Philips) to determine individual skin photo-types and to compare individual threshold doses determined in the UV-B and in the UV-A1 range. The unweighted irradiance of the UV-A1 device was measured at skin surface with a spectroradiometer (OL 754, Optronic Lab. Inc., Orlando, USA) to about 247 W m^{-2}. The exposure time to get 1 MED (250 J m^{-2}) calculated for the UV-B Erythemtester was 45 s (CIE action spectrum) and 53 s (Anders action spectrum) and differed by about 18%. In contrast, exposures with the UV-A1 device resulted in markedly larger differences. The observed exposure times to get individual erythema threshold doses varied between 28 and 55 min, depending on the individual erythema sensitivity of the volunteers which were classified into the skin photo-types I - III. This result fairly agrees with the data predicted by using the CIE action spectrum (31 min for skin type I, 39 min for skin type II and 55 min for skin type III); whereas calculations with the action spectrum of Anders et al. resulted in times between 18 min (skin type I) and 32 min (skin type III). In addition, the ratio between individual erythema threshold doses calculated with the CIE action spectrum for exposures with the UV-A1 device and with the UV-B Erythemtester differed up to about ± 20 % (skin type II) and up to about ± 40 % (skin types I and III), whereas calculations with the action spectrum of Anders et al. resulted in differences by a factor of about 3 (skin type I) and of about 1.6 (skin type III). Exposures with the UV-A1 device resulted not only in increases of the CEI but also in decreases of the ITA° which depended on exposure time and on skin type. These differences probably are due to different irradiances in the long-wave UV-A range which were used for establishing the two erythema action spectra. Whereas the LASER technique allowed irradiances of about 1500 W m^{-2} and more correlated with exposure times to get 1 MED within some minutes (Anders action spectrum), high-pressure lamps required exposure times on the order of hours (CIE action spectrum). However, exposure times on the order of 1 hour are sufficient for some repair and inducing significant immediate pigmentation which decrease erythema sensitivity just during the exposure and which cause dependence of the threshold dose on irradiance resulting in violation of the *Bunsen-Roscoe* law of proportionality. Thus, the action spectrum of Anders et al. was proved to be photobiologically correct whereas the CIE erythema action spectrum considers the effects of repair and pigmentation if the erythema threshold exposure time exceeds the need of time to stimulate significant immediate pigmentation.

OC415

Concomitant treatment of chronic plaque psoriasis with initial pulsed dye laser and narrow-band UV-B therapy

Angelika Hofer, *Daisy Kopera*, *Franz Legat J*, *Helmut Kerl*, *Peter Wolf*

Medical University of Graz / Department of Dermatology, Graz, Austria

Background: Chronic plaque psoriasis is often resistant to topical therapy and sometimes also shows a delayed response to light therapy. Recently, the pulsed dye laser (PDL) has been shown to exhibit anti-psoriatic properties by selectively damaging the microvascularization of psoriasis plaques. The purpose of this study was to investigate whether the efficacy of narrow band UV-B therapy in chronic psoriasis plaques can be enhanced by additional initial dye laser treatment.

Objectives: Eleven patients with recalcitrant plaque psoriasis on the elbows were included in the study. Three similar lesions were randomly selected to be treated either with narrow-band UV-B therapy three times a week, with PDL (585nm, 0.5msec, 7J/cm^2) once or twice at 3 week intervals or with a combination of these two treatment modalities. After 6 weeks the clinical response was

evaluated using a plaques severity score (sum score of erythema, induration, and scaling).
Results: After 6 weeks the treated plaques showed a 40%, 57%, and 68% reduction of the plaque severity score after PDL therapy, narrow-band UV-B therapy, and the combination therapy, respectively. The combined PDL/UV-B therapy was more effective compared to the PDL monotherapy (p=0.001). However, there was no significant difference between the efficacy of the combined therapy and narrow-band UV-B monotherapy.
Ten of 11 patients relapsed within 2 to 5 months. Concerning duration of remission, in 40% of the lesions there was no difference between UV-B therapy and the combined UV-B and laser therapy, in 50% it was longer in lesions treated with UV-B and laser, and in 10% it was longer in lesions treated with UVB monotherapy.
Conclusion: Concomitant treatment with initial PDL and narrow-band UV-B did not enhance the clinical outcome after 6 weeks of treatment compared to narrow-band UV-B monotherapy. However, a subset of patients may achieve a prolonged remission with the combined therapy.

OC416

Trafficking of 8-MOP treated leucocytes after photopheresis in humans

Ulrike Just[1], Elke Dimou[2], Robert Knobler[1], Gabriele Klosner[1], Erika Ivancic[1], Hildegard Greinix[3], Alexander Becherer[2], Franz Trautinger[4]
[1]General Hospital/University of Vienna, Dept. of Special and Environmental Dermatology, Vienna, Austria; [2]Dept of Nuclear Medicine, Vienna, Austria; [3]Department of Internal Medicine, Vienna, Austria; [4]Karl Landsteiner Institute for Dermatological Research, St Pölten, Austria

Photopheresis is an established treatment for cutaneous T-cell lymphoma, systemic sclerosis, graft-vs-host-disease and other autoimmune diseases. During photopheresis a leucocyte fraction (buffy coat) of the peripheral blood is extracorporeally exposed to 8-methoxypsoralen and UVA before reinfusion into the patient.
The precise mechanisms of photophesesis is not yet known. The aim of the present study was to investigate the fate of the UVA irradiated cells after reinfusion.
For this purpose a fraction of the UVA-irradiated buffy coat was sampled prior to reinfusion, separated into lymphocytes (LC) and polymorphonuclear leucocytes (PMN), and labelled with Indium-111 before being reinjected into the patient. Scans were acquired at 10min, 3h, and 24h after injection. Regions of interest (ROIs) were drawn over liver, spleen, spine, right lung and heart, for background a ROI was drawn over the m. glutaeus medius. Pixel-normalised ratios of organ to background were calculated, with correction for physical decay. Each patient was investigated separately for migration of labelled LC and PMN with an interval of 4 weeks.
Five patients (4 systemic sclerosis, 1 GvHD) have been included in the study. Using trypan blue we found that viability of 8-MOP/UVA treated LC and PMN was only minimally affected by labelling and that our technique results in a sufficiently strong signal to follow the labelled cells for 24h and probably also beyond. The most prominent difference between the migration patterns of LC and PMN that was observed in all patients is the retention of LC but not of PMN in the lungs immediately after injection within the first 3h after injection. LC on the other hand were rapidly cleared from the blood pool, while PMN showed a blood pool activity over the first 3h.
These preliminary results show for the first time that specific radiolabelling of blood cells after photopheresis in humans is feasible with a high yield and low cell damage and that 8-MOP/UVA treated LC and PMN have different and specific migration patterns.

OC417

Synergies of VEGF inhibition and photodynamic therapy in the treatment of age-related macular degeneration

Maria Fernanda Zuluaga[1], Carolina Mailhos[2], Gregory Robinson[2], David T. Shima[2], Robert Gurny[1], Norbert Lange[1]
[1]Laboratory of Pharmaceutics and Biopharmaceutics, Section of Pharmaceutical Sciences, University of Geneva - University of Lausanne, Geneva, Switzerland; [2]Eyetech Research Center, Lexington, Massachusetts, United States

PURPOSE. Photodynamic therapy (PDT) and the administration of compounds acting against vascular endothelial growth factor (anti-VEGF) are approved for the treatment of choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD). Experimental evidence that the combined use of both treatment options may improve therapeutic outcome is presented.
METHODS. Fertilized chick eggs were incubated until day 12 of embryo development (EDD12) and were treated by PDT using two different photosensitizing agents (Liposomal formulation of BPD-MA; m-THPP encapsulated in polymeric nanoparticles) and were visualized using an epifluorescence microscope. Vascular occlusion of the treated zones of the chorioallantoic membrane (CAM) was assessed by fluorescence angiography 24 and 48 hours after treatment. Alternatively, PDT-treated areas were exposed to a soluble VEGF receptor antagonist (sFlt-1) 6 hours after treatment and were analyzed.
RESULTS. Vascular occlusion in the PDT-treated areas was observed with both photosensitizers 24 hours after treatment. Reperfusion of pre-existing blood vessels and first signs of revascularization were visible 48 hours after PDT. Topical administration of sFlt-1 to the treated areas augmented occlusion and limited subsequent angiogenesis in a dose-dependent manner.
CONCLUSIONS. The combined use of PDT and of agents targeting angiogenic cytokines may synergistically improve therapeutic outcome after combined treatment in patients with CNV secondary to AMD.

IL418

Photodynamic therapy stimulates anti-tumor immunity in murine models

Michael R Hamblin[1,2], Pawel Mroz[1], Ana P Castano[1]
[1]Massachusetts General Hospiatl, Boston, MA, United States; [2]Harvard Medical School, Boston, MA, United States

Cancer is a leading cause of death among modern peoples largely due to metastatic disease. The ideal cancer treatment should target both the primary tumor and the metastases with the minimal toxicity. This is best accomplished by educating the body's immune system to recognize the tumor as foreign so that after the primary tumor is destroyed, distant metastases will also be eradicated. Photodynamic therapy (PDT) involves the IV administration of photosensitizers followed by illumination of the primary tumor with red light producing reactive oxygen species that cause vascular shutdown and tumor cell apoptosis. Anti-tumor immunity is stimulated after PDT due to the acute inflammatory response, priming of the immune system to recognize tumor-associated antigens (TAA), and induction of heat-shock proteins. The induction of specific CD8+ T lymphocyte cells that recognize major histocompatibility complex class I (MHC-I) restricted epitopes of TAAs is a highly desirable goal in cancer therapy.
We report on PDT of mice bearing different tumors that express established TAAs. We utilized several cell lines transduced with foreign antigens including b-galactosidase from bacteria (CT26.CL25), ovalbumin from chickens (EL4 E.G7), green fluorescent protein from jellyfish (RIF1-GFP). The in vivo experiments with syngeneic mice revealed that there is no difference in growth rates between wild type tumors and their transduced counterparts. With the 1mg/kg BPD, 120J/cm^2 vascular PDT regimen we cured tumors with variable effectiveness (20-100%). TAA expressing tumors were always cured more than their

wild-type counterparts. In addition we studied two mouse tumors expressing more natural TAAs. These were P815, a mastocytoma expressing a mouse cancer-testis antigen known as P1A, and TC1, a lung cancer cell line engineered to express the human papilloma virus oncogene antigen HPV E7. Strong PDT-induced immune responses were induced against TAA expressing tumors including rejection of rechallenge, and regression of distant untreated tumors. Our preliminary data indicate that this response may be mediated by cytotoxic T lymphocytes that can specifically recognize known TAA epitopes as determined by DimerX assay. We expect these studies will lead to an understanding of the relevant determinants of immune response after PDT that could be rapidly applied to patient-selection and improvement in outcome for tumor-PDT.

IL419

Local phototherapy and immunotherapy for treatment of metastatic tumors

Wei R. Chen

University of Central Oklahoma, Department of Engineering and Physics, College of Mathematics and Science, Edmond, Oklahoma, USA

It has been known that phototherapy, like photothermal and photochemical therapies, can induce immune responses in the treatment of cancer. It is expected that an active immune stimulation can synergistically enhance the immunological effects of the phototherapy. A local application of immunoadjuvant has been used as the active stimulation. Photodynamic therapy (PDT) was used with a specific adjuvant, glycated chitosan (GC), for the treatment of several different tumor models in mice; the synergistic effect through the survival of the treated tumor-bearing mice was demonstrated. The same principle was also applied in the treatment of late stage, metastatic melanoma patients using local photothermal treatment and local administration of imiquimod, a toll-like-receptor agonist. The minimal invasive local treatment was effective in controlling local metastases as well as in reducing metastases at untreated, distant sites. The method, outcome, and possible mechanism of this local photoimmunotherapy in pre-clinical and preliminary clinical studies will be presented.

IL420

Photodynamic therapy-generated vaccine: therapeutic and mechanistic insights

Mladen Korbelik

British Columbia Cancer Agency, Vancouver, B.C., Canada

Potent vaccines against cancers can be generated by treating tumor cells *in vitro* with photodynamic therapy (PDT) and using them for vaccination against tumors of the same origin. We have developed therapeutic vaccine protocols based on the injection of whole PDT-treated tumor cells, and shown with mouse models that these vaccines are effective against poorly immunogenic subcutaneously growing carcinomas. In our research with PDT vaccines we are pursuing three directions: i) further optimizing the vaccine generation and treatment procedure for achieving greater efficacy under clinically compatible protocols; ii) investigating the mechanisms of action of PDT vaccine, and using this vaccine as a tool for elucidating PDT effects in general as well as a model for dissecting the induction of antitumor immune response; and iii) exploring the potential for a combined use of PDT vaccines with other cancer treatment modalities such as radiotherapy and surgery. A finding with particular clinical ramifications is that the vaccines produced from *ex vivo* PDT-treated tumor tissue are also highly effective against established tumors. This implies that surgically removed tumor tissue can be directly used for PDT vaccines (avoiding delays and restriction with establishing first cancer cell cultures) for treating the same malignancy, which opens attractive prospects for employing cancer vaccines tailored for individual patients targeting specific antigens of the patient's tumor.

IL421

Innate cells play a critical role in enhancement of anti-tumor immunity by PDT

Sandra O. Gollnick

Roswell Park Cancer Inst, Buffalo, NY, United States

PDT of murine tumors results in regimen dependent induction of an acute local inflammatory reaction, characterized in part by rapid infiltration by innate immune cells, including neutrophils. In our study of the role of innate cells in induction of anti-tumor immunity by PDT, we show PDT regimens that induce a high level of neutrophilic infiltrate generate tumor specific primary and memory $CD8^+$ T cell responses. In contrast immune cells isolated from mice treated with a PDT regimen that induced little or no neutrophilic infiltrate exhibited minimal anti-tumor immunity. Mice defective in neutrophil homing to peripheral tissues ($CXCR2^{-/-}$ mice) or mice depleted of neutrophils were unable to mount strong anti-tumor $CD8^+$ T cell responses following PDT. Our studies indicate that neutrophils appear to be directly affecting T cell proliferation and/or survival rather than dendritic cell maturation or T cell migration. These novel findings indicate that by augmenting T cell proliferation and/or survival, tumor-infiltrating neutrophils play an essential role in establishment of anti-tumor immunity following PDT. Furthermore our results may suggest a mechanism by which neutrophils might affect anti-tumor immunity following other inflammation inducing cancer therapies. We have also shown that PDT induced $CD8^+$ T cell dependent immune responses are independent of $CD4^+$ T cell help and appear to rely upon natural killer (NK) cells. Our findings lay the foundation for the rationale design of PDT regimens that lead to optimal enhancement of anti-tumor immunity in a clinical setting. Immune enhancing PDT regimens may then be combined with treatments that result in optimal ablation of primary tumors, thus inhibiting growth of the primary tumor and controlling disseminated disease.

OC422

Targeting inflamed synovium with protease-sensitive photodynamic agents

Doris Gabriel[1], Norbert Lange[1], Marino Andres Campo[1], Veronique Chobaz-Péclat[2], Robert Gurny[1], Alexander So[2], Nathalie Busso[2]

[1]Department of Pharmaceutics and Biopharmaceutics, University of Geneva, Geneva, Switzerland; [2]CHUV-Laboratoire de Rheumatologie, Lausanne, Switzerland

Background: Photodynamic therapy has been proposed as a minimally invasive alternative to surgical, chemical and radioisotopic ablation of chronically persistent inflammation in small distal joints of rheumatoid arthritis patients. We currently aim to refine photodynamic treatment in this indication by the use of a non-phototoxic, protease-sensitive polymeric prodrug (PPP). Tissue-specific restoration of PPP phototoxicity is achieved by targeting enhanced proteolytic activity within the inflamed synovium. Thrombin plays a crucial role in ongoing inflammation during RA, and significant up-regulated activity makes this serine protease a promising candidate for tissue specific activation of a protease-sensitive PPP.

Purpose: The present study was designed to determine the optimal conditions for the specific activation of these photosensitizer prodrugs in diseased synovia, thus leading to selective photodestruction in arthritic tissue.

Material and methods: Specific activation of the thrombin-sensitive PPP (T-PPP) by the target enzyme was shown by analytical HPLC analysis. In vitro phototoxicity of the non-activated and pre-activated T- PPP was tested in vitro on human synoviocytes (RASFs) obtained from rheumatoid arthritis patients. The intracellular localization of PPPs was studied by confocal fluorescence microscopy. In vivo pharmacokinetics and prodrug activation were elucidated in a collagen-induced arthritis (CIA) mouse model, by fluorescence imaging, confocal fluorescence

microscopy of tissue sections and fluorimetric HPLC analysis of tissue extracts.
Results: Analytical HPLC analysis confirmed cleavage of the pheophorbide-peptide and PPPs by thrombin. Cellular phototoxicity on RASFs was shown to be higher following in vitro predigestion in comparison to the non-digested PPP. Confocal fluorescence microscopy of PPP incubated RAFS revealed punctuated cytoplasmatic distribution of PPPs. In vivo, fluorescence imaging of living mice and tissue sections revealed a significant increase in PS-fluorescence and homogeneous distribution within inflamed tissues. Finally, PS-peptidyl fragments extracted from tissue homogenates underline the dual targeting strategy of passive accumulation of the macromolecular carrier within the inflamed synovium and subsequent active enzymatic release of phototoxic PS peptidyl-fragments.

OC423

Photodynamic therapy induces immunity against a beta-galactosidase expressing tumor

Pawel A Mroz, Michael R Hamblin
Department of Dermatology Harvard Medical School, Wellman Center for Photomedicine Massachusetts General Hospital, Boston, MA, United States

Photodynamic therapy can stimulate anti-tumor immunity against cancer by increasing expression of cytokines and promotion of immune recognition of the tumor cells. PDT may also increase the immunogenicity of dead tumour cells by exposing or releasing of tumor associated antigens (TAA). Identification of TAA recognized by CD8+ T cells and the corresponding major histocompatibility complex class I (MHC-I) restricted epitopes was a groundbreaking step in cancer immunology. TAA-specific $CD8^+$ T cells represent an important component of the host's immune response against malignant diseases.
The goal of this study was to investigate application of PDT to an established TAA model. Lacking a well defined murine TAA, we utilized a β-galactosidase (β -gal, a bacterial protein) transduced colon tumor cell line CT26.CL25 that stably expressed β-gal as well as its class I MHC restriction element $H\text{-}2L^d$ syngeneic to BALB/c mice. PDT with a regimen of 1mg/kg BPD IV, and 120 J/cm^2 690-nm after 15 minutes successfully cured all CT26.CL25 tumors with 100% effectiveness, while all CT26 wild type tumors showed local recurrence. After 90 days of tumor free interval the mice cured from CT26.CL25 were rechallenged and remained resistant. The CT26.CL25 cured mice did not reject CT26 wild type tumor cells. Experiments with mice bearing two CT26.CL25 tumors (one in each leg) and only one tumor treated with PDT, showed that the immune response was strong enough to induce regression of the distant untreated tumor in 70% of the cases. Mice bearing two CT26 wild type tumors, and only one treated with PDT, demonstrated progression of both tumors in all cases. T lymphocytes from cured mice recognized the immunodominant peptide of β-galactosidase and were also able to specifically lyse CT26.CL25 cells in a 51Cr release assay. These data show for the first time that PDT can, in addition to locally curing tumors, induce epitope specific anti-tumor immune response capable of destroying distant, well-established tumors expressing model tumor antigens.

IL424

Advances in antimicrobial photodynamic therapy

Michael R Hamblin[1,2], George P Tegos[1], Aguinaldo S Garcez[3]
[1]Massachusetts General Hospital, Boston, MA, United States; [2]Harvard Medical Schools, Boston, MA, United States; [3]Centro de Laser e Aplicações, IPEN-CNEN/SP, Sao Paulo, Sao Paulo, Brazil

The relentless increase in multi-antibiotic resistant microbes, and fears that much infectious disease, formerly thought to be trivial, will eventually become "untreatable" has led to the search for alternative methods of killing pathogens and treating infections. Although PDT killing of bacteria has been known for over a hundred years, its use to treat infections has not been much developed. We have studied a range of highly active antimicrobial PS and developed synergistic combinations that dramatically enhance the efficacy of antimicrobial PDT. We have tested new combination treatments with photosensitizers and with non-toxic adjuvant substances to potentiate the photodynamic inactivation of pathogenic microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Proteus mirabilis*. Low dose hydrogen peroxide (1μM to 1 mM) and both natural and synthetic inhibitors of multi-drug efflux pumps were tested in combination with antimicrobial photosensitizers of various chemical structures. Surprising and significant synergy was observed between low dose hydrogen peroxide and PDI mediated by multiple different photosensitizers. From ten times to one hundred times more killing could be achieved when illumination was carried out in a concentration of hydrogen peroxide that was otherwise non-toxic. Non-toxic multi-drug inhibitors could synergistically increase the PDI killing of both Gram-positive and Gram-negative bacteria mediated by phenothiazinium dyes.
We present a new "bench-to-bedside" application of antimicrobial PDT. A polycationic photosensitizer conjugate between polyethyleneimine and chlorin(e6) (PEI-ce6) was designed and synthesized and proved highly affective in photoinactivating multiple classes of pathogen. Using bioluminscent reporter bacteria we showed that root canals in ex vivo human teeth could be effectively disinfected by a combination of mechanical endodontic therapy and PEI-ce6-mediated PDT. A clinical trial of PEI-ce6 PDT and 660-nm laser light delivered by fiber optic into root canals in 20 patients undergoing endodontic therapy demonstrated a highly significant increase in the antibacterial effect. As further basic studies into the mechanims of antimicrobial PDT are made and more effective antimicrobial photosensitizers are developed we believe that the number of clinical applications of the technology will further increase.

IL425

Photosensitisers for antimicrobial PDT: physico-chemical and photobiological properties

Giulio Jori
Department of Biology, University of Padova, Padova, Italy

A photosensitiser with potentially optimal properties for the treatment of microbial infections should be endowed with specific features in addition to the expected photophysical characteristics. Such features include: a large affinity for microbial cells; a broad spectrum of action (namely a cytocidal activity against bacterial, fungal and protozoan pathogens) in order to efficiently act on infections involving a heterogeneous flora of pathogens; a mechanism of cell photoinactivation minimizing the risk of inducing the selection of resistant strains or promoting the development of mutagenic processes; and the possibility to identify a therapeutic window which allows (a) the extensive killing of the disease-inducing microbial cells with minimal damage to the host tissue in the area of infection and (b) the prevention of the regrowth of the pathogens after the photodynamic treatment. The available evidence points out that optimal photoantimicrobial properties are typical of photosensitising agents that are positively charged at physiological pH values (e.g., for the presence of quaternarized amino groups or the association with polylysine moieties) and are characterized by a moderate hydrophobicity (n-octanol/water partition coefficient around 8). In general, quite satisfactory results have been obtained by using photosensitisers belonging to the families of phenothiazines, chlorins, porphyrins and phthalocyanines. These photosensitisers in a micromolar concentration can induce a 5-6 log decrease in the microbial population after incubation times as short as 5-10 minutes and irradiation under mild experimental conditions, such as fluence-rates around 50 mW/cm^2 and irradiation times shorter than about 15

minutes. Further enhancement of the photoantimicrobial activity can be achieved by introducing relatively long alkyl chains at the periphery of the photosensitiser molecule or binding the photosensitiser to peptides which enhance the selectivity of microbial cell targeting, as well as the penetration to inner cell districts.

IL426

Potential of PDT for control of parasitic infection

Ross William Boyle[1], *Timothy Paget*[2], *Carrie-Anne Bristow*[1]

[1]University of Hull, Kingston-upon-Hull, E. Yorkshire, United Kingdom; [2]Universities of Kent and Greenwich at Medway, Chatham, Kent, UK

Protozoan parasites of the genus Leishmania are the causative agents for a number of serious human diseases including cutaneous and mucocutaneous Leishmaniasis. These diseases affect 12 million people worldwide and control of Leishmaniasis remains a problem due to the lack of effective vaccines, drug resistance and the paucity of useful drugs. PDT is a promising treatment modality for Leishmaniasis as resistance is less likely to occur, due to the multiple mechanisms of cytotoxicity involved. Preliminary clinical studies are currently underway.

The aim of this study was to screen a range of cationic porphyrins for differential activity against L.major and L.mexicana promastigotes, keratinocytes and macrophages. From a small library of compounds we identified one which showed significant inhibition of L.major and L.mexicana promastigotes without significant toxicity to macrophages and keratinocytes. Three other porphyrins showed ability to kill Leishmania promastigotes and macrophages without significant toxicity to keratinocytes. Uptake studies in promastigotes showed localisation of some porphyrins within the kinetoplast, an organelle specific to parasites of the order Kinetoplastida. Our results indicate that PDT could be used to treat cutaneous and mucocutaneous Leishmaniases and it is feasible to develop porphyrins that exhibit differential activity, thus limiting damage to surrounding tissue.

IL427

Light-activated antimicrobial agents for the treatment of periodontitis - a clinical evaluation

Michael Wilson

University College London, London, United Kingdom

Periodontitis is the most prevalent chronic infectious disease of humans and is caused by the accumulation of bacterial biofilms (dental plaques) at the gingival margin. Antimicrobial agents are used in the prevention and treatment of the disease but concern over the emergence of antibiotic-resistant microbes has lead to the search for alternative antimicrobial approaches. Photodynamic Therapy (PDT) is an example of one possible approach. *In vitro* studies have shown that the organisms responsible for periodontitis can be killed by red light in the presence of low concentrations of a number of photosensitisers. Most importantly, killing could be achieved in the presence of serum and blood and when the organisms were grown as biofilms. In a recent clinical study involving 33 adults with periodontitis, PDT was carried out using 0.005% (w/v) methylene blue and irradiation with 670 nm light from a 150 mW diode laser. The energy dose per site was 10-20 $J.cm^{-2}$. Three clinical regimens were evaluated using a total of 622 periodontal sites: (i) mechanical debridement (MD) alone, (ii) PDT alone and (iii) PDT + MD. Clinical assessments of bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL) were made at baseline, 3 weeks and 12 weeks following therapy. After 12 weeks, the PDT + MD group exhibited statistically significant improvements of 239% in CAL and 150% in PPD compared to the MD group. The results of this clinical study have shown that PDT can significantly enhance the effectiveness of the currently-used mechanical approaches to the treatment of periodontitis.

IL428

A double-blinded, randomised, placebo controlled clinical trial to determine whether PDT using the phenothiazinium salt, PPA 904 can reduce bacterial load in chronic leg ulcers and chronic diabetic foot ulcers

S.B. Brown[1], *C. O'Grady*[2], *K. Mellish*[2], *J. Griffiths*[1], *H. Moseley*[3], *M. Rizwan*[4], *L.E. Rhodes*[4], *S.M. Morley*[3]

[1]Centre for Photobiology and Photodynamic Therapy, University of Leeds, Leeds, UK; [2]Photopharmica Limited, Leeds, UK; [3]Ninewells Hospital and Medical School, University of Dundee, Dundee, UK; [4]University of Manchester and Salford Royal University Hospital, Manchester, UK

Chronic leg ulcers and chronic diabetic foot ulcers can be of very long duration (months to years), can seriously impair the quality of life of sufferers and, in the case of diabetic foot ulcers, can lead to life-threatening conditions. They also represent a severe financial burden on health care services. There is evidence that bacterial levels in the ulcer in excess of 10^5 colony forming units (cfu) per cm^2 can inhibit healing. This presentation reports the first controlled clinical trial using antimicrobial PDT in chronic ulcers. The purpose of the trial was to determine if PDT using the phenothiazinium derivative 3,7-bis(N,N-dibutylamino) phenothiazin-5-ium bromide (PPA904) can reduce the bacterial colonisation of these chronic ulcers. 32 patients (16 with leg ulcers and 16 with diabetic foot ulcers) with bacterial colonisation in excess of 10^5 cfu/cm^2 were recruited for the study. Drug or placebo was applied topically followed by red light. Patients were given a single treatment of either drug plus light or placebo plus light.

The trial primary end point was to determine if PDT can reduce the bacterial levels of chronic ulcers in patients receiving PPA 904 and light, compared with patients receiving placebo and light. The trial succeeded in reaching its primary end point, demonstrating a statistically significant reduction in bacterial load in ulcers of patients on active drug compared with those on placebo ($p = 0.011$). For the diabetic foot ulcer group analysed separately and for the leg ulcer group analysed separately, in each case there was a statistically significant decrease in bacterial load for patients on active drug ($p<0.001$), but there was no significant decrease in patients on placebo.

The secondary end point was to see if there was any evidence for improved healing in those patients receiving active therapy compared with those on placebo, though the trial was not powered to demonstrate significance in healing. For the leg ulcer group, 4 of 8 patients on active drug (50%) achieved complete healing, whereas only 1 of 8 patients (12.5%) achieved complete healing in the placebo group. In the diabetic foot ulcer group, insufficient patients completed three months of study to permit statistical analysis.

Further analysis showed that several types of bacterial species were killed in these treatments including MRSA. The treatment was found to be safe and well-tolerated by patients. No treatment-related adverse events were reported.

OC429

Enzyme-activated photosensitizers for use in antimicrobial photodynamic therapy

Sarika Verma, *Ulysses Sallum*, *Humra Athar*, *Gregory Watt*, *Tayyaba Hasan*

Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States

The continuing emergence of clinical pathogens that are resistant to many or even all standard antimicrobial chemotherapeutics provides the necessary impetus to develop alternative technologies. Radically new approaches are required to overcome this problem of

microbial resistant mutants. We propose a novel strategy wherein the production of beta-lactamase enzymes, the very basis of a resistance mechanism, is exploited not only for enhancing the photochemical killing of drug resistant bacteria but also for the specificity of bacterial killing.

Our strategy revolves around developing microbial-specific light activatable drugs called photosensitizers (PS) for use in photodynamic therapy against microbial infections. Molecular design focuses on the development of new constructs that are effectively known as pro-PS (as in pro-drugs). These pro-PS are inactive (with or without light) while linked as a construct and are light activatable only when released by the beta-lactamase enzyme mediated cleavage, providing us with the unique opportunity to target beta-lactamase producing (drug-resistant) bacterial cells. Considering that many clinical isolates abundantly produce beta-lactamase at infectious sites, such targeted photodynamic therapy should greatly limit the degree of photodamage to host tissue and thus enhance treatment efficacies. Therefore in the proposed strategy, unlike conventional antibiotics, where hydrolysis of the beta-lactam ring by beta-lactamase inactivates the antibiotic, the lactam ring opening of the pro-drugs will release the PS and make it light activatable for photokilling.

Various PS are being synthesized and investigated for the antimicrobial photodynamic therapeutic effects on both Gram-negative and Gram-positive bacteria such as *E. coli* and *S. aureus*. This presentation will focus on the conjugate design and establishment of the most effective pro-PS construct following biological testing.

OC430

The green line of frequency doubled Neodymium:YAG Laser for killing the sensitized pathogenic bacteria in comparison with the red light of He:Ne Laser

Siham A Kandela[1], *Alic K Melconian*[2]
[1]*Al-nahrain University, College of Science, Baghdad, Iraq;* [2]*Baghdad University, College of Science, Baghdad, Iraq*

Low power frequency doubled Nd: YAQ laser of green emission (532 nm) is used for inhibition of methicillin resistant *Staphylococcus epidermidis* (MR *S. epidermidis*) in conjugation with thionin and azure agents. Spectroscopic study of seven sensitizers revealed that thionin and azure can be efficient for photodynamic action (PDA) when activated with the laser green line to produce total killing effect of the above under study bacteria. Azure can be also efficient with red light of He:Ne laser but not thionin. Total killing was obtained using thionin sensitizer at concentration 75µg/ml irradiated by the green radiation of dose 3.36 J/cm^2 and at concentration of 100 µg/ml if irradiated with 1.12 J/cm^2 dose. Total killing occurred also with azure agent at concentration of 75 µg/ml and 100 µg/ml when irradiated with 4.48 J/cm^2 and 3.36 J/cm^2 respectively.

Results with He:Ne laser revealed, as expected, total killing when azure is used at concentration of 50 µg/ml and 75 µg/ml and irradiated with laser red light of 9.6 J/cm^2 and 7.2 J/cm^2 respectively.

Results with He:Ne laser revealed, as expected, total killing when azure is used at concentration of 50 µg/ml and 75 µg/ml and irradiated with laser red light of dose 9.6 J/cm^2 and 7.2 J/cm^2 respectively. Thionin agent yield no perfect killing neither within the sensitizers concentration range nor within irradiation dose range adopted; it produced high killing percentage of 98.7% at maximum concentration (100 µg/ml) using maximum laser dose(9.6) J/cm^2. These results demonstrate that laser green line has better efficacy in killing MR *S. epidermidis* than does laser red light when the same sensitizers concentration (thionin or azure) are used with both wavelengths. The procedure followed proved to be an efficient way to determine the inhibition percentage accurately.

IL431

Forced proliferation of epidermal stem and progenitor cells with accumulated UV-induced DNA damage gives rise the foci of cells overexpressing wild type p53

Joanne GW Nijhof, Leon HF Mullenders, Frank R de Gruijl
Leiden Univ Med Ctr, Leiden, Netherlands

Chronic low-dose UV exposure of hairless mice causes some basal cells in the epidermis to accumulate cyclobutane pyrimidine dimers (CPDs). The frequency of these CPD-retaining basal cells (CRBCs) is dependent on the DNA repair proficiency of the mouse (Ddb2-/- > Ddb2+/- > Ddb2 +/+ > Tg-Ddb2); 6-4 photoproducts do not accumulate, apparently signifying a rate of repair that exceeds the rate of induction. All BrdU-retaining stem cells in the interfollicular epidermis showed accumulation of CPDs, as did MTS24+ progenitor cells in the upper part of hair follicles. To investigate whether the CRBCs that encompass stem and progenitor cells could become oncogenic foci, we applied TPA (12-O-tetradecanoylphorbol-13-acetate) to force these cells into proliferation. The CRBCs disappeared after 1 – 2 weeks of TPA (twice weekly), and after 4 weeks many foci of cells overexpressing p53 occurred. In contrast to ‘p53 patches’ induced by chronic high-dose UV exposure, these TPA-induced foci did not express p53 in mutant conformation, and their frequency (about 1 for every 4 CRBCs) was too high to be attributable to gene mutation. The focal nature of wild type p53 overexpression suggests that the daughter cells of the CRBCs may show delayed stress, possibly by compounded genetic instability. Carcinogenic implications of these TPA-induced wt p53 foci need to be investigated further.

IL432

Protein Kinase C epsilon interacts with Stat3 and regulates its activation to sensitize skin to ultraviolet radiation-induced development of squamous cell carcinomas

Ajit K Verma
Department of Human Oncology, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin, USA

Chronic exposure to the sun’s ultraviolet radiation (UVR) is linked to the development of human squamous cell carcinoma (SCC), the metastatic nonmelanoma skin cancer. We have reported that PKCε transgenic mouse lines 224 and 215, which overexpress PKCε protein approximately 8- and 18-fold, respectively, over endogenous levels in the basal epidermal cells and cells of the hair follicle, are highly sensitive to UVR-induced development of SCC (Cancer Resaerch 64, 7756-7765, 2004). To find clues about the mechanisms by which PKCε sensitizes skin to UVR carcinogenesis, we found that PKCε physically interacts with Stat3, phosphorylates Stat3Ser727 and increases both DNA-binding and transcriptional activity of Stat3. Constitutively activated Stat3 is linked to the development of SCC. Several lines of evidence support the link between PKCε and Stat3. First, both PKCε and activated Stat3 are overexpressed in SCC. Second, using reciprocal immunoprecipitation/blotting experiments, it was observed that PKCε associates with Stat3. Finally, double immunofluorescence studies confirm their co-localization. Also, in *in vitro* immunocomplex kinase assays, PKCε phosphorylated Stat3 at serine 727 residue. Inhibition of PKCε using PKCε specific siRNA: 1) attenuated Stat3Ser727 phosphorylation, decreased DNA-binding and transcription activity of Stat3, 2) decreased expression of Stat3-regulated genes. We conclude that PKCε activation is an initial signal in the activation of Stat3, which is linked to induction, progression of SCC. UVR-induced phosphorylation of Ser727 may be a key component of the mechanism by which PKCε imparts sensitivity to UVR- induced development of SCC.

IL433
Blocking serotonin and/or platelet activating factor receptor binding interferes with photocarcinogenesis
Stephen E. Ullrich
The University of Texas, MD Anderson Cancer Center, Houston, Texas, USA
The ultraviolet (UV) radiation found in sunlight is a major cause of human skin cancer. In addition, UV exposure is immune suppressive and UV-induced immunosuppression is a major risk factor for skin cancer induction. Recently we discovered that two of the very early steps in the UV-induced pathway of events leading to immune suppression are: 1) Binding of *cis*-urocanic acid to the serotonin ($5HT_{2A}$) receptor. 2) Binding of the lipid mediator of inflammation, platelet-activating factor (PAF) to its receptor. Here we tested the hypothesis that selective serotonin or PAF receptor antagonists would block skin cancer induction. Hairless mice were irradiated with solar simulated UVB radiation (1.25 kJ/m^2) on Monday, Wednesday and Friday. Immediately prior to irradiation, some of the mice were injected with a PAF receptor antagonist or a $5HT_{2A}$ receptor antagonist. Skin cancer induction was then monitored. Compared to the UV-irradiated controls, treatment with either the PAF or $5HT_{2A}$ receptor antagonists decreased tumor incidence and increased tumor latency. When sub-optimal doses of the drugs were used, injecting the PAF or $5HT_{2A}$-receptor antagonist alone had no protective effect, but when used together they synergized to suppress skin cancer induction. We also observed that the PAF and $5HT_{2A}$ receptor antagonists blocked the severity of UV damage to the skin as measured by the inhibition of UV-induced apoptosis, UV-induced induction of sunburn cells and UV-induced induction of reactive oxygen species. These data indicate that PAF and $5HT_{2A}$ receptor antagonists have the potential of being developed into novel anti-cancer agents.

IL434
Topical tacrolimus in combination with simulated solar radiation does not enhance photocarcinogenesis in hairless mice
Catharina M. Lerche[1], Peter A. Philipsen[1], Thomas Poulsen[2], Hans Christian Wulf[3]
[1]Department of Dermatology D92, Bispebjerg University Hospital, Copenhagen NV, Denmark; [2]Department of Pathology, Soenderborg Sygehus, Soenderborg, Denmark; [3]Department of Dermatology D42, Bispebjerg University Hospital, Copenhagen NV, Denmark
Numerous studies have demonstrated the utility of topical tacrolimus in atopic dermatitis. However, there is a concern that local immunosupression by calcineurin inhibitors may enhance dermal photocarcinogenesis. Therefore, we investigated the influence of topical tacrolimus on squamous cell carcinoma formation in hairless female C3.Cg/TifBomTac immunocompetent mice exposed to solar simulated radiation (SSR).
In a first experiment mice (n=200) had tacrolimus applied on their dorsal skin 3-times weekly followed by SSR (2, 4, or 6 standard erythema doses, SED) 3-4 hours later. Tacrolimus did not reduce the time to tumor development and in the group receiving 4 SED it even had a protective effect (156 days versus 170 days, p=0.008).
In a second experiment mice (n=50) were irradiated with 6 SED 3-times weekly for 3 months and subsequently treated 5-times weekly with topical tacrolimus to mimic the use of tacrolimus on sun-damaged skin. Median time to the first skin tumor was 234 days in SSR+tacrolimus group compared to 227 days in the only SSR-irradiated group (p=0.160).
In a third experiment mice (n=25) had tacrolimus applied on their dorsal skin every day for 1month whereafter the group was irradiated with 4 SED 3-times weekly. Median time to the first skin tumor was 142 days in tacrolimus + SSR group compared to 156 days in the only SSR-irradiated group from experiment one (p=0.363).
We conclude that tacrolimus does not accelerate photocarcinogenesis in hairless mice.

OC435
UV-induced carcinogenesis in a p53 mutant mouse model: increased sensitivity to skin damage by a point mutation at codon 172
Cara L. Benjamin, Chengming Zhu, Honnavara N. Ananthaswamy
UT M.D. Anderson Cancer Center, Department of Immunology, Houston, TX, USA
The p53 tumor suppressor gene is involved in numerous pathways including DNA repair, regulation of cell cycle, apoptosis, and ultimately plays a role in carcinogenesis. Genetic disorders such as Li-Fraumeni Syndrome involve mutations of the p53 gene and result in predisposition to early onset of tumors, including skin cancers. Previous work on UV-carcinogenesis in a p53-null mouse model resulted in increased tumor formation demonstrating the importance of p53 in protecting against UV-induced cancer. To define the role of p53 in UV response, we utilized a mouse model with a hot spot mutation at codon 172 in the DNA binding region of the p53 gene (R172P) to investigate the role of p53 mutations and UV-induced skin cancer. P53 R172P animals were irradiated three times per week with a bank of six unfiltered FS40 sunlamps. The p53 mutant mice displayed hypersensitivity to UV-radiation compared to p53-null and wild-type animals resulting in increased skin damage, which coincides with *in vitro* data that R172P mouse embryonic fibroblasts were also hypersensitive to UVR. Chronic UV-irradiation at low doses (2 kJ/m^2/exposure) resulted in shorter life span in R172P homozygous and heterozygous mutant animals over the wild-type littermates and non-irradiated control mice. A high incidence of internal tumors was also observed in the R172P homozygous population that resulted in early mortality of these animals. Interestingly, exposure of R172P heterozygous mice to low doses of UV radiation resulted in development of squamous cell carcinomas and fibrosarcomas much earlier and at a high frequency than wild-type mice. Genetic analysis of tumors did not reveal loss of the remaining mutant p53 allele, but they contained alterations in the CDKN2A gene at a high frequency. These results indicate that the $p53^{R172P}$ mutation results in an increased sensitivity to UV-irradiation, and that in the presence of the mutation the wild-type allele cannot protect from UV-induced skin cancer.

OC436
Deregulation of the cell death response to UVB and cisplatin in squamous cell carcinoma cell lines
Sofie Claerhout[1], An Van Laethem[2], Lien Verschooten[1], Charlotte Proby[3], Patrizia Agostinis[2], Marjan Garmyn[1]
[1]Lab of Dermatology, University of Leuven, Leuven, Belgium; [2]Division of Biochemistry, University of Leuven, Leuven, Belgium; [3]Centre for Cutaneous Research, University of London, London, United Kingdom
The UVB fraction of solar radiation is a complete carcinogen and apoptosis of UVB-damaged keratinocytes (sunburn cells) is a fail-safe mechanism, which eliminates potentially mutagenic keratinocytes. Circumventing the apoptotic program is an acquired trait of many tumour cells. In addition, resistance to chemotherapy appears to be associated with apoptosis deficiency of tumour cells in many cases. Insight into the biological behaviour of the different stages of squamous cell carcinoma of the skin and the molecular mechanisms leading to resistance to chemotherapeutic drugs is a requisite to find effective anticancer therapeutics. In previous work, we showed that in UVB-irradiated primary human keratinocytes, AKT activation delays the onset of mitochondrial apoptosis, while the ASK-1/p38MAPK pathway plays a key role in the onset of apoptosis after UVB irradiation. In this study, key protein kinase-driven cell death and survival pathways activated

following exposure to UVB irradiation or to the chemotherapeutic drug cisplatin, were studied in the squamous cell carcinoma (SCC) cell lines representing different stages of malignant transformation of the epidermis: PM1 (cultured from dysplastic forehead skin), MET1 and MET4 (respectively derived from a primary SCC and its regional lymph node metastasis). We observed that while the MET4 cells displayed the highest resistance to UVB-induced apoptosis, a phenotype coupled with a disregulation of the UVB induced pro-apoptotic p38 MAPK pathway, the primary SCC MET1 cells were more sensitive to UVB-induced apoptosis than the premalignant PM1 cells. Inhibition of the PI3-K/AKT pathway by wortmannin increased sensitivity to UVB-induced apoptosis in all cell lines, thus implicating this pathway as a major survival signal following UVB irradiation. Although UVB and cisplatin are two genotoxic cellular stresses, a marked difference in apoptosis resistance and in the morphological phenotype associated, was observed following UVB or cisplatin treatment. SCC cells in the further stages of carcinogenesis (e.g. MET1 and MET4) were pronouncedly more resistant to apoptotic cell death following cisplatin treatment than UVB irradiation. In conclusion, these results show that the cell death response to UVB is deregulated in SCC cell lines and that this response differs from the one to cisplatin. We are further investigating the mechanism underlying the difference in the apoptotic response to UVB and cisplatin in these SCC cell lines.

OC437

Changes in epigenetic regulation of chromatin in human epidermis and primary human skin cells after UV-irradiation *in vivo* and *in vitro*

Ruediger Greinert, Kristina Behrend, Beate Volkmer
Dermatologisches Zentrum Buxtehude, Buxtehude, Germany

Epigenetic modifications like DNA methylation and/or histone methytlation and acetylation play important roles in regulation of gene expression and in maintenance of certain chromatin structures that have been found to be altered in cells of various tumors. Epigenetic patterns have to be changed or restored during processes like DNA replication and repair and are essential for the control of stemness in embryonic or adult stem cells as well as for processes of differentiation. Therefore, the investigation of epigenetic alterations is of central interest in cancer research.

We were interested wether UV-irradiation of human skin or primary human skin cells in vivo and in vitro is able to change epigentic patterns in irradiated skin and might be related to skin cancer.

Therefore we irradiated human skin in vivo with solar simulated UV-radiation (SSUV) and primary human skin cells in vitro with different qualities of UV-radiation (UVA: 315- 400 nm and UVB: 280-315 nm) and investigated histone methylation dose- and time-dependently. H3K4 methylation (as a marker for euchromatin) and H3K9 methylation (as a marker for heterochromatin) were measured by means of fluorescence microscopy using monoclonal antibodies against the methylated histones.

Our results show, that UVA and UVB irradiation changes histone methylation patterns of human skin cells. Most importantly, we've been able to show that in vivo SSUV irradiation induces a more heterochromatinic pattern in basal cells (keratinocytes and melanocytes) of human skin epidermis which have persistently accumulated cyclobutane pyrimidine dimers (CPD retaining basal cells, CRBCs) compared to undamaged cells. This effect was more pronounced in CRBCs of keratinocytic than of melanocytic origin. We discuss wether these changes in epigenetic pattern is a result of UV-induced damage in these cells or characterizes the possible stem cell nature of these cells.

IL438

Exploiting plant UV responses for sustainable crop production in a changing climate

Nigel D. Paul, Jason M. Moore, Jason J. Wargent
Department of Biological Sciences, Lancaster University, Lancaster, Lancashire, UK

The last thirty years has seen huge strides in understanding how plants respond to UV radiation. Research driven by concerns over increasing UV-B due to ozone depletion has clarified the range of responses, with changes in plant morphology (e.g. reduced stem elongation and leaf area, increased leaf thickness) and chemistry (e.g. increases in flavonoids) being among the most consistent. It is also clear that these responses are not a function only of UV-B-doses above the ambient range, variation within the ambient range also induces significant effects. The increasingly availability of commercial horticultural plastics with contrasting UV transmission properties offers a route to the practical exploitation of these UV responses. We have investigated the scope for this "technology transfer" by growing a wide range of horticultural crops under commercial high tunnels, covered with plastics with contrasting UV transmissions. In general, growing crops under contrasting UV environments produces the type of response expected from more fundamental research. For example, in almost every crop we have studied the harvested biomass declines with increasing UV transmission. However, this greater biomass often comes at the cost of poorer crop quality, for example in terms of taste or colour. A response of particular commercial interest has been the effects of UV on crops such as lettuce, cabbage, cauliflower and broccoli which, in the UK, are often propagated under protection for 3-6 weeks before being transplanted to their final position in the field. Growers have clear criteria for what constitutes a good-quality at the end of propagation, including compact growth and good mechanical strength. We hypothesised that these criteria would be delivered by propagation under a plastic that was largely transparent to solar UV. This was confirmed experimentally. Compared with plants propagated in the glasshouse or under "conventional" plastics, those propagated under a UV-transparent (UV-T) plastic were significantly shorter, had significantly reduced leaf area, but significantly increased leaf thickness. These plants not only met the commercial criteria for high quality, they also performed substantially better after being transplanted to their final field positions. The final yield from plants propagated under UV-T were up to 40% higher than those propagated under standard commercial conditions. Initial investigations of the mechanisms of this improved performance are suggesting that they can be explained by UV-responses that have been well-defined in previous, more fundamental research.

IL439

Interaction of UV-B radiation and low temperature: damage and acclimation

Wolfgang Bilger, Karena Hoffmann-Wülfing, Matthias Schultze
University of Kiel, Kiel, Germany

Epidermally located UV screening compounds, mainly flavonoids and hydroxycinnamic acid derivatives (HCAs), serve as a protection of plants against UV-B radiation. The biosynthesis of the screening compounds is regulated by UV-B radiation, but also by other environmental factors. We have shown that temperature can regulate the synthesis of flavonoids in a wide variety of plants. In the presentation it will be dealt with two main questions: How does low temperature induce biosynthesis even in the absence of UV-B radiation, and why is this adaptive for a plant? A prerequisite for the action of low temperature is the presence of light, which in itself enhances flavonoid biosynthesis in an irradiance dependent manner. The induction of quercetin glycosides depends on the interaction of blue light with low temperature, defining a new role for a blue light sensor as an important lead in a temperature sensing signal transduction chain. As a model system for UV-B-induced

damage, photosystem II will be used. We show that low temperature increases PS II damage by inhibiting the recovery from damage. Acclimation to low temperature increases the resistance of plants and we investigate the contribution of epidermal screening to this resistance increase. It is concluded that low temperature is an important environmental factor in modulating UV-B damage in plants and their defence of this damage.

IL440

Flavonoids and iridoids in plants: their role and responses to environmental change

Ken G Ryan[1], *Rilka Taskova*[1], *Allan Burne*[1], *Lisa Bryant*[1], *Rod Seppelt*[2], *Phil Garnock-Jones*[1]

[1]*Victoria University of Wellington, Wellington, New Zealand;* [2]*Australian Antarctic Division, Hobart, Tasmania, Australia*

Abiotic stresses in plants give rise to the generation of toxic reactive oxygen species (ROS) that attack cell macromolecules. Plants normally develop specific enzymes and low molecular weight antioxidants to quench these ROS and to prevent oxidative damage. Here we examine two groups of secondary metabolites that protect plants from ROS damage.

High levels of flavonoids in leaves may provide resistance to UV-B damage because of their antioxidant properties. We have shown previously that di-hydroxylated flavonoids are better ROS scavengers than their mono-hydroxylated counterparts and are preferentially synthesised under high Ultraviolet-B (UV-B) fluxes. Depletion of stratospheric ozone over Antarctica since the mid 1970s has led to significant increases in UV-B radiation. Herbarium samples of the Antarctic moss *Bryum subrotundifolium* spanning periods before and after the formation of the ozone hole were analysed by HPLC. Total flavonoid concentrations increased as ozone levels reduced (and UV-B levels increased). Furthermore, the concentration of the di-hydroxylated flavonoid luteolin increased, while the mono-hydroxylated apigenin did not change. This provides a natural verification of the timing of the onset of ozone depletion over Antarctica and reflects associated increases in UV-B radiation.

Iridoids may have originated as bitter compounds that protect plants from grazing. Our recent observations show that iridoids are produced in enormous concentrations in the New Zealand *Veronica* complex, yet they have no apparent anti-grazing properties. Furthermore, while these plants are exposed to high levels of UV-B throughout New Zealand, they have very low levels of flavonoids. Unlike most iridoids, our recent assays reveal that the *Veronica* iridoids have significant and sometimes very high antioxidant activity. Iridoids in *Veronica* are unusual because they utilize a precursor compound from the flavonoid biosynthetic pathway that confers antioxidant properties to the iridoid. We suggest that during the evolution of the *Veronica* complex in New Zealand, these iridoids have taken over the antioxidant function of flavonoids by coopting precursors otherwise destined for flavonoid production. As a result of this work we need to re-assess the current hypotheses of the role of iridoids and the evolution of secondary metabolites function in plants.

OC441

Plant response action spectra in the spot-light

Emma McLoughlin[1], *Kevin Newsham*[2], *John Moncrieff*[1], *Andrew McLeod*[1]

[1]*Institute of Atmospheric and Environmental Sciences, School of GeoSciences, Edinburgh University, Edinburgh, UK;* [2]*British Antarctic Survey, Natural Environment Research Council, Cambridge, UK*

Since the discovery of the ‘ozone hole’ over Antarctica there has been continued research into the biological effects of increased UV-B (280-315 nm) irradiances at the Earth’s surface caused by stratospheric ozone depletion. The biological effects of UV-B are wavelength dependent and several spectral sensitivity functions, otherwise known as action spectra, have been developed for predicting various biological responses to different wavelengths of UV.

Recently, Flint & Caldwell (2003) proposed a new action spectrum, the UV plant growth weighting function, which shows biologically effective UV extending into UV-A wavelengths. Commonly-used action spectra in previous plant investigations showed minimal, if any, effectiveness of UV-A wavelengths. Because of the effectiveness of UV-A wavelengths, the radiation amplification factor (RAF) for the new weighting function is lower than that of the many commonly-used action spectra. A lower RAF implies less change in UV-B irradiance, supplied from lamps, per unit change in ozone column. From this, in another article Flint & Caldwell (2003) concluded that previous experiments have applied a lower dose of UV-B than was necessary to simulate a given level of ozone reduction, and that previous data would need to be re-evaluated to determine the magnitude of UV-B effects on plants.

Here we present a modelling study in which we compare the amount of biologically effective radiation weighted with commonly-used action spectra (Green et al. [1974] and Thimijan et al. [1978]) with that of Flint & Caldwell (2003). We find little difference between the action spectra in the amount of radiation applied from lamps to simulate a given level of ozone depletion.

OC442

Effect of UV-B light and temperature on primary photosynthetic processes in two lichen species from contrasting habitats

Hana Vrablikova[1], *Per Larsson*[2], *Kristyna Vecerova*[1], *Knut-Asbjorn Solhaug*[2], *Yngvar Gauslaa*[2]

[1]*Institute of Experimental Biology, Department of Plant Physiology and Anatomy, Faculty of Science, Masaryk University, Brno, Czech Republic;* [2]*Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, Aas, Norway*

UV-B light (280-320 nm) is an important stress factor for all photosynthetic organisms as they are dependent on solar radiation. Lichens often occur in environments with high solar radiation and therefore with high UV-B light. One of the most efficient photoprotective mechanisms of lichens against UV-B light is a synthesis of secondary metabolites, e.g. melanins (*Lobaria pulmonaria*) and parietin (*Xanthoria parietina*). Our aim was to test the influence of different UV-B levels on primary photosynthetic processes of the two lichen species with different susceptibility to high light – *X. parietina* grows in open habitats, while *L. pulmonaria* is the shade-adapted species.

For 15 days, we exposed lichen thalli to 4 different levels of UV-B light (from 0 to 1 W m^{-2}) in two temperature-controlled rooms at 12 °C and 21 °C. Photoperiod in both rooms was 18 h/6 h, with photosynthetically active radiation intensity of 200 μmol m^{-2} s^{-1}. The UV-B light was applied for 16 hours during the light period. The primary photosynthetic processes of thalli were analyzed by chlorophyll fluorescence measurements by means of slow curve supplemented with saturation pulses.

The exposure to different intensities of UV-B light had nearly same negative effect on the maximum quantum efficiency of PSII (Fv/Fm) of lichen thalli. However, non-photochemical quenching of chlorophyll fluorescence (NPQ) was highest in thalli exposed to highest UV-B intensity. Similarly, the amount of melanins (*L. pulmonaria*) and parietin (*X. parietina*) in lichen thalli increased with raising intensity of UV-B light. Finally, dry thalli were exposed to 1000 μmol m^{-2} s^{-1} for 72 hours in order to test their high light susceptibility. Lichen thalli exposed previously to UV-B light were more resistant to photoinhibition of photosynthesis induced by high light.

The first author’s participation on this project was supported by travel grant of European Society for Photobiology.

PL501

DNA repair from past to future

Leon H.F. Mullenders

Department of Toxicogenetics, Leiden University Medical Center, Leiden, The Netherlands

The small fraction of UV-B radiation in sunlight at the earth surface is predominantly accountable for the deleterious effects of solar radiation including skin cancer. Direct absorption of solar UVB leads to the formation of the toxic and mutagenic photolesions i.e. cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts. Nucleotide excision repair (NER) is a highly conserved repair system and in humans the principal pathway for removal of sunlight induced photolesions. In addition to base excision repair, NER might also work on some types of oxidative DNA damage induced by UV radiation. During the last decades we have gained a fascinating picture of the steps and the components involved in NER, the regulation of the process and the biological consequences of defective NER in animal models and in man. In my presentation I will focus on the NER reaction (carried out by a multiprotein complex) and discuss a variety of aspects: the molecular mechanisms covering the initial step of DNA damage recognition up to final restoration of chromatin, the activation of NER by DNA damage signalling, the impact of mutations in key genes in this pathway as manifested in UV sensitive human syndromes, the role of NER subpathways in controlling acute and long term hazardous effects of sunlight, the interplay between NER and bypass of replication blocking photolesions, differences in NER activity between rodent and human and between different types of skin cells. Finally I will address outstanding questions and future directions of research.

IL502

Breast characterization and lesion detection by means of optical spectroscopy and imaging

Paola Taroni[1], Daniela Comelli[1], Antonio Pifferi[1], Lorenzo Spinelli[1], Alessandro Torricelli[1], Gianmaria Danesini[2], Rinaldo Cubeddu[1]

[1]Politecnico di Milano, Milan, Italy; [2]Casa di cura S. Pio X, Milan, Italy

Time-resolved transmittance and reflectance spectroscopy allows the non-invasive *in vivo* assessment of the absorption and scattering properties of breast. The absorption properties provide information on tissue composition and the scattering properties on its structure. Up to four main absorbers are typically involved in this interpretation of tissue absorption data, namely the two forms of hemoglobin, water and lipids. Collagen has never been considered up to now. However, it is one of the main components of breast tissue and seems to be involved in cancer development. The quantification of collagen content in breast could also be of interest for the non-invasive classification of breast density, again related to the risk of developing cancer. Taking this into account, we have measured the optical properties of collagen and we are now working to investigate the diagnostic potential of collagen quantification.

Based on the information derived from spectroscopy, we have also developed the first optical mammograph operating in the time domain at 4 to 7 wavelengths between 635 and 975 nm (red to near infrared). Absorption and scattering images are collected in compressed breast geometry, deriving information on tissue composition. The instrument was tested in a clinical trial on 200 patients with malignant and benign lesions, allowing us to identify optical features that characterize the different breast structures (*e.g.* mammary gland, blood vessels, adipose regions) and lesion types. Detection rates for cancers and cysts in one view are 96% and 90%, respectively, while they decrease to 80% and 83%, if detection in two views is required. Correlation between demographic parameters (*e.g.* age, body mass index) and tissue composition and structure, as derived from optical data, was investigated. Based on the results achieved, an upgraded version of the instrument, with extended spectral capabilities to allow for collagen quantification, has recently been developed and is now entering a new clinical trial.

IL503

Optical signatures for ovarian cancer screening

Urs Utzinger[1], Molly Brewer[2]

[1]Biomedical Engineering, Optical Science, Obstetrics and Gynecology, University of Arizona, Tucson, Arizona, USA; [2]Gynecologic Oncology, University of Connecticut, Farmington, Connecticut, USA

Among gynecologic cancers, ovarian cancer is the second most common and has the highest mortality. Currently no accurate early diagnostic technique for ovarian cancer. Furthermore, little is understood regarding the early progression of this disease. Optical signatures arising from endogenous tissue constituents can be measured spectroscopically and offer potential for diagnostic and therapeutic information. Results from our latest studies indicate that optical signatures might be able to identify ovaries at increased risk for developing cancer. We have developed a clinical measurement device compatible with laparoscopic procedures allowing us to further study the ovary's optical signature. Ultimately minimally invasive endoscopic techniques will allow the screening of women at increased risk for developing cancer or will serve as follow-up procedures for abnormal serum tests.

We will also present results from multiphoton microscopic imaging of ovarian tissue, illustrating significant structural alterations of the stroma and changes in the redox ratio of the epithelial lining during carcinogenesis and in patients at increased risk.

IL504

Differential Pathlength Spectroscopy for medical diagnosis

H.J.C.M. Sterenborg, B. Kruijt, S. Kaskakova, , H.S. de Bruijn, D.J. Robinson, A. Amelink

CODT Erasmus MC, Rotterdam, The Netherlands

Optical biopsy for non-invasive diagnosis of cancer is an ongoing challenge to biomedical optics. Extensive clinical studies on the measurement of optical properties of tissue using spatially resolved steady-state diffuse reflectance spectroscopy revealed that human tissues often are too inhomogenous for diffusion theory to work accurately enough for reliable clinical diagnosis. Differential Pathlength Spectroscopy is a recently developed technique that measures optical properties of turbid tissues more locally and has since been evaluated on optical phantoms and for various clinical applications. On the basis of these local measurements we can determine the volume, saturation and average vessel diameter of the microvasculature as well as the local light scattering properties of the tissue. In vivo studies during bronchoscopic diagnosis of lung cancer on 120 patients revealed that these parameters could be used for diagnosis and were even relevant to the type and stage of the disease. In addition a one year follow up of the patients whowed that the microvascular saturation was indicative of the patient's survival. In recent studies we expanded this to measurements on Barrett's esophagus, oral cancer, and intraoperatively to breast cancer and brain tumours. In addition, we recently started to use DPS measurements for investigating changes in the microvasculature during Photodynamic Therapy. The presentation will explain the optics of the approach and present the latest experimental results.

OC505

***In vivo* imaging of the bronchial and alveolar walls microstructure using fibered confocal autofluorescence microscopy**

Genevieve Bourg-Heckly[1], Luc Thiberville[2], Sophie Moreno-Swirc[2], Christine Vever-Bizet[3]
[1]Universite Pierre et Marie Curie Paris 6, CNRS UMR 7033, Paris, France; [2]Hopital Charles Nicolle CHU Rouen Clinique Pneumologique, Rouen, France; [3]Universite Pierre et Marie Curie Paris 6, BioMoCeTi CNRS UMR 7033, Paris, France

Objectives: To analyse the microscopic autofluorescence structure of bronchial and alveolar mucosae using fibered confocal fluorescence microscopy (FCFM).
Methods: The dual fibered confocal fluorescence imaging and spectroscopic system used here is developed in collaboration between Mauna Kea Technologies and our laboratory, the BioMoCeTi. It is based on a flexible 1.4 mm diameter confocal miniprobe composed of 30000 fiber cores, used for the fluorescence excitation (488 nm) and the generation of the microscopic images. The frame rate reaches 12 images/sec for a 600x500 μm field of view with an image lateral resolution of 3.5 μm; the spectral resolution is 3 nm. Spectrum and image acquisitions are synchronized.
Results: Two clinical trials are conducted in the Hôpital Charles Nicolle-CHU (Rouen):
1) Autofluorescence microspectro-imaging of bronchi: FCM was used to analyse in vivo the microscopic autofluorescence structure of the normal and pathologic bronchial mucosae during bronchoscopy. The first autofluorescence microscopic images and associated spectra ever carried out in patients were obtained. Ex vivo and in vivo microscopic and spectral analyses showed that the signal mainly originates from the elastin component of the subepithelial layer (basement membrane zone) of the bronchial wall. In the normal bronchi, reproducible images were obtained, characterized by a highly organized fibered network. Conversely, 19/22 dysplasia or metaplasia, 5/5 carcinoma in situ and 2/2 invasive lesions exhibited an alteration of this fibered network.
2) Autofluorescence microspectro-imaging of alveoli: The Alveoflex miniprobe small size allows to explore bronchi down to alveoli. In non-smokers, the procedure produced the first real time in vivo images and spectra of alveoli originating from the interstitial elastic backbone. In active smokers, a specific in vivo imaging of the alveolar macrophages and alveolar walls was obtained in relation to tobaco tar fluorescence.

OC506

Development of microcystoscopy for the characterization of lesions detected by fluorescence imaging with Hexvix® in the human bladder

Blaise Lovisa[1], Patrice Jichlinski[2], Daniela Aymon[2], Hubert van den Bergh[1], Georges A. Wagnieres[1]
[1]Swiss Federal Institute of Technology (EPFL), CH 1015 Lausanne, Switzerland; [2]CHUV University Hospital, CH 1011 Lausanne, Switzerland

The simultaneous presence of multiple tumors and/or the presence of occult flat tumors such as high grade dysplasia and carcinoma in situ have an important impact on the prognosis of superficial bladder cancer. Therefore, a sensitive and specific minimally invasive detection of these lesions, which are often invisible in white light cystoscopy, is of high importance. Our long-term research in this context aimed at optimizing the selective production and accumulation of protoporphyrin IX (PPIX) from topically applied derivatives of aminolevulinic acid (ALA) within cancerous tissues. The analysis of the PPIX accumulation kinetics and the measurement of its distribution across the urothelium enabled us to select the optimal type of ALA derivative and its administration conditions. The combined efforts of in vitro, preclinical and clinical studies in Lausanne, in which the hexylaminolevulinate (Hexvix®) was selected, led to the phase II and III clinical trials undertaken together with Photocure ASA, and finally to the approval of this detection procedure in 27 European countries. FDA approval procedure has also been initiated. Although the selective production of PPIX and the sensitivity of this procedure are outstanding, its specificity is limited due to false positive lesions that are mainly associated with inflammations of the bladder mucosa. Therefore, our current research focuses on the improvement of the specificity of this detection method. New methods, using high magnification endoscopy, are being investigated by our group in order to discriminate false from true positive findings, and hopefully resulting in a reduced number of biopsies. In this study, we are using a dedicated magnification cystoscope, allowing conventional magnification with “macroscopic” white light and fluorescence observation, as well as image acquisition with high magnification (HM) when the endoscope is in contact with the tissue. This is realized by an optical setup directly integrated in the cystoscope. The field of view of the obtained image is 500 microns in the HM mode and the resolution is about 3 microns. With this optical setup, the on going study is aimed at observing and characterizing the neo-vascularization of the flat lesions, which will help to distinguish (pre-)cancerous tissue from inflammation. Thus, we are trying to define some independent criteria, such as vessel tortuosity, increase of the microvascular density and vessel diameter. This will allow us to significantly improve the fluorescence cystoscopy method described above. Preliminary clinical results will be presented.

OC507

***In vivo* optical properties of melanin and non-invasive detection of melanoma**

George Zonios[1], Aikaterini Dimou[1], Ioannis Bassukas[1], Dimitrios Galaris[1], Efthimios Kaxiras[2]
[1]University of Ioannina, Ioannina, Greece; [2]Harvard University, Cambridge, USA

We present a new method for the non-invasive study of melanin optical properties *in vivo* which is based on diffuse reflectance spectroscopy of human skin. We have observed that the optical absorption spectrum of melanin exhibits an exponential dependence on wavelength *in vivo*, which is consistent with *in vitro* results. We offer theoretical justification for this exponential dependence on the basis of a recently proposed model for the structure of eumelanin protomolecules. In addition, we demonstrate intrinsic differences in absorption spectra between malignant melanoma and dysplastic nevi, by analyzing our own clinical data as well as data from three independent, previously published studies. We find that the histologic transition from dysplastic nevi to melanoma in situ and then to malignant melanoma is clearly reflected in the melanin absorption spectra. Our analysis indicates that *in vivo* investigation of the melanin light absorption properties can be a powerful tool for noninvasive detection of melanoma, as well as for the study and characterization of pigmented skin lesions. It is also a promising approach for deeper understanding of the biological role, structure, and function of melanin.

OC508

Correlation between PPIX fluorescence and both tissular effects and pain induced by PDT on normal skin using fluorescence imaging

Jérôme Barge[1], Thomas Glanzmann[2], Hubert van den Bergh[1], Georges Wagnières[1]
[1]Photomedicine Group, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland; [2]Photoderma SA, Lausanne, Switzerland

Introduction: Outcome, adverse events as well as pain induced during topical PPIX-PDT are subject to significant inter- and intra-individual variations in current dermatological practice. We investigated the correlation between PPIX fluorescence intensity

and both the tissular effects (erythema) and the pain induced by PDT, on normal skin, after topical administration of aminolevulinic acid (ALA) derivatives.
Methods: Photosensitization tests were performed on the normal skin of the legs of 5 volunteers with various drug doses (active concentration 10% to 100%; cream 6 mg/cm^2) and formulations of diethyleneglycol-monoethylether aminolevulinate (ALA-DGME), a PPIX precursor showing an optimal selective photosensitization of the pilosebaceous unit. Circular spots (3 cm in diameter) were homogeneously illuminated at 635 nm with a frontal light distributor coupled to a diode laser. The irradiances, light doses and drug-light intervals (DLI) ranged between 50 and 200 mW/cm^2, 10 and 200 J/cm^2, and 1 and 6 hours, respectively. The PPIX fluorescence above 665 nm was quantified using a filtered camera, exciting at 635 nm in order to probe "deep seated" PPIX. The evolution of erythema was assessed by reflectance spectroscopy between 1 and 8 weeks after PDT. The pain was scored during illumination using a numerical scale ranging from 0 to 10.
Results and conclusion: A correlation exists between the PPIX fluorescence measured on normal skin before PDT and both the tissular effect and the pain induced at a given irradiance and drug-light interval. Major inter- and intra-patient variations are observed for PPIX fluorescence, indicating that the variations in PPIX concentration are probably at least in part responsible for the major fluctuations in pain, outcome or adverse events encountered in current PPIX-PDT practice in dermatology.

OC509

Fluorescence dynamics studies of a PDT photosensitizer

Muhammad Atif
Pakistan Institute of Engineering and Applied Sciences, Islamabad, Punjab, Pakistan

Experiments carried out using a Titanium Sapphire laser delivering trains of ~ 130 fs duration pulses at a wavelength of 800 nm to excite fluorescence in mTHPC via a two-photon absorption step. The high irradiance available with this laser led to relatively efficient two-photon excitation, allowing fluorescence from the photosensitizer to be readily detected using a fiber based system. Fluorescence results are reported for mTHPC incorporated in solvent (PEG-ethanol). A preliminary assessment of the photodynamic activity induced by the two-photon excitation route is made, based on the measurements of the viability of photosensitized epithelium cells, following exposure to various dose levels at 800 nm. The results are in excellent agreement with Schneider *et al.*[1] investigation.

[1]Schneider M et al. Proc. SPIE, "Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in Photodynamic Therapy IX", 2000, 3909, 60-65.

OC510

Synthesis and photophysical study of fluorescent bile acid derivatives suitable for anion transport measurements on hepatocytes

Maria Luisa Marin[1], Jana Rohacova[1], Miguel Angel Miranda[1], Maria José Gómez-Lechón[2], José Vicente Castell[2]
[1]Instituto de Tecnología Química-Universidad Politécnica de Valencia, Valencia, Spain; [2]Centro de Investigación, Hospital Universitario La Fé, Valencia, Spain

Bile acid transport involves uptake from the blood and subsequent excretion to the bile canaliculi. Cholestasis is the failure of bile to reach the duodenum. In this communication development of novel fluorescent derivatives as probes for bile acids and anion transport, to be used in assays for detection of cholestasis, is reported. New fluorescent derivatives of natural bile acids have been synthesized, and their suitability as substrates of relevant transport systems by hepatocytes has been assessed by means of direct kinetic assays using simultaneously fluorescent analogs and natural non-fluorescent substrates of the transporters. The bile-acid analogs have an emission wavelength of ~520 nm, and their transport inside the cell can be detected and quantified by measuring the increase of green fluorescence in cells after exposure to these bile-acid derivatives. The flow cytometry assay allows to discriminate between live and dead cells, and analyze the uptake of the bile acid derivatives only in the former. Also, the real-time kinetics of bile-acid derivatives uptake can be evaluated using time as a cytometric parameter. Two different sorts of fluorescent derivatives have been prepared: first, a fluoresecein derivative of the cholic acid, was chosen as the fluorophore. Then, a smaller fluorophore (4-nitrobenzo-2-oxa-1,3-diazol (NBD)) was chosen to synthesize analogs substituted at positions C-3 and C-7. N-Fluoresceinyl-3α,7α,12α-trihydroxy-5β-cholan-24-oic amide was prepared from the cholic acid in three stages. First, protection of the hydroxyl groups, then formation of the amide between the carboxylic acid and fluorescein, and final deprotection of the hydroxyl groups. In the case of NBD a synthetic methodology that allows the derivatization of the cholic acid by positions 3α, 3β, 7α or 7β has been developed. This methodology is based on the selective transformation of the desired hydroxyl group into an amino function. Once the amino group is formed, derivatization using NBD-Cl and deprotection of the methyl ester is accomplished in two more steps. Thus, four NBD-derivatives have been prepared. In order to validate the specificity of the uptake of the fluorescent derivatives, troglitazone, a bile-acid uptake inhibitor is used. Best results have been obtained with NBD-derivatives, especially for 3α and 7α.

IL511

Oxygen 18-labeled singlet molecular oxygen as a valuable tool for studying DNA damage

Paolo Di Mascio[1], Glaucia R. Martinez[2], Marisa H.G. Medeiros[1], Didier Gasparutto[3], Jean-Luc Ravanat[3], Jean Cadet[3]
[1]Departamento de Bioquímica - Instituto de Química - Universidade de São Paulo, São Paulo, Brasil; [2]Departamento de Bioquímica e Biologia Molecular – Setor de Ciências Biológicas - Universidade Federal do Paraná, Curitiba, Paraná, Brasil; [3]Laboratoire Lésions des Acides Nucléiques, DRFMC-SCIB-E n. 3 CEA-UJF, CEA-Grenoble, Grenoble, France

Cellular components, including unsaturated fatty acids, proteins, and DNA, are potential targets to oxidation reactions by singlet molecular oxygen ($^1O_2$). We have recently synthesized the water-soluble ^{18}O-labeled endoperoxide of *N,N'*-di(2,3-dihydroxypropyl)-1,4-naphthalene dipropanamide (DHPN$^{18}O_2$) generating high yields of ^{18}O-labeled singlet oxygen $^{18}[^1O_2]$. Oxidation products thus formed in the presence of such a generator of $^{18}[^1O_2]$ should be labeled with, at least, one ^{18}O atom. Guanine is the only normal nucleic acid base that significantly reacts with $^1O_2$ in the $^1\Delta_g$ state at neutral pH. The main reaction was found to be a Diels-Alder [4 + 2] cycloaddition of $^1O_2$ across the 4,8-bond of the imidazole ring of guanine producing unstable 4,8-endoperoxides. The two main decomposition products of $^1O_2$-mediated oxidation of 2'-deoxyguanosine (dGuo) in aqueous solutions were the two diastereomers of spiroiminodihydantoin (dSp) nucleosides. A relatively minor product of the $^1O_2$ oxidation of free dGuo has been found to be 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo). Interestingly, the formation of 8-oxodGuo becomes predominant at the expense of the dSp nucleoside in the presence of reducing agents such as thiols or Fe^{2+} ions. A similar situation is found in double-stranded DNA since only the formation of 8-oxodGuo has been detected. Interestingly, it was shown that 8-oxodGuo, an exposure marker of DNA to most oxidizing agents is a much better substrate than dGuo to further oxidation by $^1O_2$. The rate of reaction of $^1O_2$ with 8-oxodGuo is about two orders of magnitude higher than that with dGuo. It is likely that $^1O_2$ adds across the 4,5-ethylenic bond of 8-oxodGuo to generate a transient dioxetane. This leads to the predominant formation of cyanuric acid together

with 2,2,4-triamino-5-(2*H*)-oxazolone and dSp nucleosides. The reaction of $^1O_2$ with an 8-oxodGuo residue, site-specifically inserted within a single-stranded oligonucleotide, was found to be more specific. Thus, the predominant oxidation product in this system was identified as oxaluric acid and subsequent reactions yielding the formation of oxaluric acid through the parabanic acid precursor. Additional information on the mechanism may be obtained using a clean chemical source of ^{18}O-labeled $^1O_2$, $DHPN^{18}O_2$, and additional labeling experiments with $H_2{}^{18}O$. The products thus formed can be detected and quantified using the accurate HPLC-ESI-MS/MS method.

Financial support: FAPESP, CNPq, CNPq-Instituto do Milênio Redoxoma.

IL512

Mechanisms of cellular damage induced by peptide- and protein-peroxides generated by photooxidation

Michael J. Davies

The Heart Research Institute, Sydney, NSW 2050, Australia

Proteins are major biological targets for oxidative damage within cells due to their high abundance and their rapid rates of reaction with both a wide range of free radicals and a number of excited state species including singlet oxygen. Exposure of proteins to singlet oxygen has been shown to generate peroxide species in high yield, with these species formed predominately on Tyr, Trp, and His residues. The precise chemical structures of some of these materials have been elucidated. Protein peroxides have been detected on both isolated proteins and on proteins within intact cells on exposure to a photosensitizer and light. These peroxides decompose in the presence of reducing agents, UV light, heat, and metal ions but are long-lived in the absence of these agents. Reaction with metal ions generates further radicals that can oxidise other biomolecules resulting in chain reactions within proteins, induction of strand breaks and mutagenic lesions in DNA, inhibition of cellular enzymes, altered cellular redox status, and antioxidant depletion. Amino acid and peptide hydroperoxides can be reduced by some cellular enzymes (e.g. glutathione peroxidase) and low-molecular-mass species such as GSH and ascorbate. Removal of protein hydroperoxides, both enzymatically and by low-molecular-mass species is however inefficient, and this is believed to underlie their long half-lives within cells. A major target of these species is GSH and thiol residues on proteins, with the latter potentially resulting in enzyme inhibition. In this presentation the role of peroxides generated on peptides and proteins by singlet oxygen in inactivating key thiol-dependent enzymes (e.g. GAPDH, caspases, cathepsins and protein tyrosine phosphatases) will be reviewed. These studies demonstrate that peroxide formation on one protein can result in subsequent selective damage to other proteins, with enzymes with low pK_a Cys residues particularly sensitive targets. In many cases the extent and rate of enzyme inactivation is greater than that observed with H_2O_2. In the case of thiol-dependent cathepsin enzymes these reactions result in the inactivation of the systems that are designed to remove damage proteins. We have hypothesized that this results in vicious cycle that results in the accumulation of damaged proteins within cells. In the case of protein tyrosine phosphatases inactivation potentially results in cellular and tissue dysfunction by modulation of phosphorylation-dependent cell signalling in systems subject to oxidative stress.

IL513

UVA sensitivity in Smith-Lemli-Opitz Syndrome: possible involvement of cholesta-5,7,9(11)-trien-3β-ol

Colin F. Chignell, Robert H. Sik, Piotr J. Bilski, Yu-Ying He, Barbara M. Kukielczak

Laboratory of Pharmacology & Chemistry, NIEHS/NIH, Research Triangle Park, NC, USA

Smith-Lemli-Opitz Syndrome (SLOS) is a severe developmental disorder caused by mutations in the *DHCR7* gene coding for 7-dehydrocholesterol (7-DHC) reductase, the enzyme involved in the last step of cholesterol biosynthesis. SLOS homozygotes exhibit a marked deficiency of cholesterol in plasma and tissues with a concomitant increase in 7-DHC. UVA photosensitivity has been reported in ~60% SLOS patients with maximal response occurring at 350 nm (Anstey et *al*. Br. J. Dermatol. 141, 406, 1999). 7-DHC itself has no UVA absorption and so cannot be the direct cause of SLOS photosensitivity. However, cholesta-5,7,9(11)-trien-3β-ol (9-DDHC), a metabolite of 7-DHC that has strong absorption in the UVA range (ϵ ~ 15,000 @ 324 nm), has been detected in plasma from SLOS patients (De Fabiani et *al*. J. Lipid Res. 37, 2280, 1996) and may be involved in SLOS photosensitivity. We have detected both 7-DHC and 9-DDHC in skin lipids extracted from CD-1 mice treated with AY9944 (*trans*-1,4-bis(2-chlorobenzylaminomethyl)-cyclohexane), an inhibitor of 7-DHC reductase. Human HaCaT keratinocytes treated with 9-DDHC and then immediately exposed to UVA exhibited decreased viability when compared to dark controls. No damage was observed in cells exposed to 7-DHC/UVA or UVA alone. 9-DDHC was detected in keratinocytes incubated with 7-DHC for 15 h; these cells were damaged when exposed to UVA. We have found that conversion of 7-DHC to 9-DDHC may occur by either Type I or II mechanisms and have identified a common intermediate, namely 7-hydroperoxy-5,8(9)-cholestatriene-3beta-ol, which decomposes to give 9-DDHC and hydrogen peroxide. UVA irradiation of 9-DDHC in acetonitrile generates superoxide and carbon-centered and alkoxyl radicals which are trapped by 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). Singlet oxygen is generated when 9-DDHC is UVA irradiated in CCl_4. Reactive oxygen species are detected in 9-DDHC/UVA exposed keratinocytes using the fluorescent probe 5-(and 6-)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester. These findings suggest that reactive oxygen species generated by 9-DDHC could play a role in the UVA skin photosensitivity of SLOS patients (Chignell et *al*. Free Rad. Biol. Med. 41, 339, 2006).

IL514

Role of cardiolipin peroxidation in apoptotic cell death induced by mitochondrial photooxidative damage

Albert W Girotti[1], Witek Korytowski[1,2], Tamas Kriska[1]

[1]Medical College of Wisconsin, Milwaukee, Wisconsin, United States; [2]Jagiellonian University, Krakow, Poland

Cardiolipin (CL), an acidic phospholipid with four unsaturated fatty acyl chains, is located exclusively in the mitochondrial (Mito) inner membrane (IM), where it interacts with cytochrome c (cyt c) and other proteins, and is required for optimal respiratory activity. Model studies with CL in thin film or liposomal form have shown that its normally tight association with cyt c is progressively weakened with increasing CL peroxidation, i.e. conversion to hydroperoxide (CLOOH) species. Cyt c dissociation from the IM and release into the cytosol for apoptosome activation is now recognized as a key event in the intrinsic pathway of oxidative stress-induced apoptosis. CLOOH formation under these conditions could be a major determinant of cyt c mobilization; recent evidence suggests that cyt c itself can promote this by acting as a CL peroxidase. In the present study, we examined CL's involvement in apoptosis photosensitized by 5-aminolevulinate-induced protoporphyrin IX localizing in Mito. Two hypotheses were put forward: (1) Cyt c is mobilized when its tethering CL is photoperoxidized; (2) The CLOOHs themselves are mobilized and translocate to the outer membrane (OM) for recruitment of pore-

forming apoptogenic proteins (tBid, Bax). Support for (1) was obtained by showing that a tumor line transfectant clone overexpressing the Mito-localizing form of GPx4, a LOOH detoxifying selenoperoxidase, was much more resistant to photoinduced CLOOH accumulation, cyt c release, caspase-3 activation, and apoptotic death (observed in this temporal order) than a vector control. Support for (2) derived from experiments showing that CLOOHs undergo lipid transfer protein-stimulated migration from IM- to OM-mimetic liposomes, permitting tBid/Bax targeting and permeabilization of the latter. tBid recruitment increased progressively with CLOOH content of acceptor membranes, CLOOH being much more effective in this regard than unoxidized CL. When other unoxidized or peroxidized phospholipids were used (e.g. PC or PCOOH), tBid binding was minimal by comparison. These findings provide new insights into the unique role of CL oxidation in Mito-launched photodynamic apoptosis.

Supported by NIH: CA72630

OC515

Adaptive responses to singlet oxygen generated stress in *Rhodobacter*

Jens Glaeser[1], Monica Zobawa[2], Friedrich Lottspeich[2], Gabriele Klug[1]

[1]Justus Liebig Universitaet, Institut fuer Mikrobiologie und Molekularbiologie, Giessen, Germany; [2]Max Planck Institut fuer Biochemie, Martinsried, Germany

Singlet oxygen ($^1O_2$) is a stress factor and signal in the facultative phototrophic bacterium *Rhodobacter sphaeroides*. *In vivo* protein labeling with L-[^{35}S]-methionine and analysis by 2D gelelectrophoresis revealed that synthesis of 61 proteins was changed in response to $^1O_2$. Obtained protein synthesis patterns were distinct from H_2O_2 and similar to high light exposure. This finding indicates that regulatory mechanisms are specific for $^1O_2$ and that the response to light is partly mediated by $^1O_2$. Our results suggest that the response to $^1O_2$ is regulated mainly by *rpo*E (σ^E) but a response independent from σ^E was observed.[1] Proteins affected in synthesis rate by singlet oxygen were grouped into three different regulatory categories reflecting different responses. Previously a sigma factor cascade of σ^E and *rpo*H_2 (σ^{32}) was suggested, because the promoter region of σ^{32} contained a σ^E target sequence.[2] However, analysis by 2D gelelectrophoresis revealed that σ^{32} is not involved in the regulation soluble cytoplasmic and periplasmic proteins upon singlet oxygen exposure. Alternative regulatory mechanisms in response to singlet oxygen mediated photoxidative stress may very likely involve small regulatory RNAs in *Rhodobacter*, as indicated by altered protein synthesis patterns in a strain lacking the RNA chaperone *hfq*.

[1]Glaeser et al. (2007) Journal of Proteome Research (in press); [2]Braatsch et al. (2004) Journal of Bacteriology 186 (22) 7726-7735.

IL516

Photoreceptor genes in the fungus *Phycomyces blakesleeanus*

Catalina Sanz[1], Julio Rodriguez-Romero[2], Alex Idnurm[3], Victor G. Tagua[2], Luis M. Corrochano[2], Joseph Heitman[3], Arturo P. Eslava[1]

[1]Universidad de Salamanca, Salamanca, Spain; [2]Universidad de Sevilla, Sevilla, Spain; [3]Duke University, Durham, NC, USA

The fruiting body of the fungus *Phycomyces blakesleeanus* is sensitive to many environmental signals, including light, gravity, and the presence of nearby objects. Blue light changes the speed and direction of growth of the fruiting body resulting in positive phototropism. The isolation of mutant strains with altered phototropism allowed the identification of several *mad* loci. Phototropism and other light responses are defective in *madA* and *madB* mutants suggesting that their corresponding gene products play relevant roles in *Phycomyces* photobiology. We have recently shown that the *madA* gene product in similar to the *Neurospora* protein WC-1, a LOV-domain photoreceptor and transcription factor. In *Neurospora* WC-1 interacts with WC-2, another Zn-finger transcription factor, to form a WC complex that binds the promoters of light-regulated genes, presumably to activate gene transcription upon photon reception. The *Phycomyces* genome contains three *wc-1* genes and four *wc-2* genes that would allow the formation of many different WC complexes that may be optimized to operate with different light intensities or in specialized cellular locations. In addition, *Phycomyces* has a cryptochrome gene similar to those involved in plant blue light reception. We are currently sequencing the *Phycomyces* photoreceptor genes in the *mad* mutants aiming to identify those that are relevant for photoreception. We have found that the *madB* mutant has a mutation in one of the *wc-2* genes, *wctA*, that prevents the correct splicing of an intron suggesting that the *madB* gene is a *wc-2* homolog. The co-segregation in genetic crosses of the blind phenotype and the mutation in the *wctA* gene confirmed that the *madB* mutant has a mutation in the *wctA* gene. The splicing mutation results in a short MADB protein without the Zn-finger, presumably preventing the correct operation of the MAD complex. The transcription of some of the *Phycomyces wc* genes are regulated by light. The mRNAs for *madA* and *madB* accumulate in the dark, suggesting that the corresponding MAD complex is present in dark-grown mycelia ready to activate transcription upon photon reception. The remaining *wc-1* genes, *wcoA* and *wcoB*, and two of the *wc-2* genes, activate their transcription after light exposure, suggesting that the corresponding gene products might be involved in long-term photoreception. We have measured the threshold of light-dependent gene transcription and found different sensitivities for the activation of each gene, perhaps reflecting the use of specialized MAD complexes for light-dependent activation in each *wc* gene.

IL517

Structural and functional studies of the *Bacillus subtilis* blue-light sensor YtvA and related proteins

Aba Losi

University of Parma, Department of Physics, Parma, Italy

Bacterial LOV (Light, Oxygen and Voltage) proteins related to the plant blue-light receptors phototropin (phot), are photochemically active with the photoinduced transient formation of a covalent adduct between the flavin chromophore and a conserved cysteine residue. In bacteria, the photoswitchable LOV domain is associated to diverse protein modules, e.g. histidine kinases (HK), phosphodiesterases, response regulators (RR), transcriptional activators. Besides their intrinsical interest as novel blue-light sensors, these modular systems can therefore help in elucidating a key question in the field of LOV-based sensing: which are the molecular basis of light-to- signal tranduction from the LOV core to the effector domain? In this view, we are presently investigating three LOV systems: the B. subtilis protein YtvA, built of a LOV and STAS (Sulphate Transporter, AntiSigma factor antagonist) domain, the “ short LOV” protein SB2 from P. putida and the hybrid LOV-HK-RR protein from the plant pathogen P. syringae. In YtvA the STAS domain can bind triphosphate nucleotides (NTP) and light-driven conformational changes are transmitted from the LOV core to the NTP-binding cavity. Mutation of a conserved residue in the central beta scaffold of the LOV core, abolishes this signal transmission, highlighting a key role of this protein surface during light sensing and a trasnmitter function for the linker region, two features previously suggested also for plant phot. Accordingly, conformational analysis indicates that the LOV-STAS interface largely overlaps with the LOV-LOV dimerization surface, mostly formed by the central beta scaffold. In SB2, a short LOV proteins with no associated effector modules but with a helical sequence C-terminal to the LOV core (analogous to the linker region), aromatic residues belonging to the central beta sheet undergo light-driven

conformational changes and interact with the helical C-cap. In the P.syringae LOV-HK-RR kinase, self-phosphorylation is enhanced under light conditions, similar to plant phot, but the molecular surface of the LOV core involved in this process remains to be characterized.

IL518

Spectroscopic and structural characterization of a plant member of the cryptochrome DASH subfamily

Richard Pokorny[1], *Julia Moldt*[1], *Tobias Klar*[2], *Lars-Oliver Essen*[2], *Alfred Batschauer*[1]

[1]*Philipps-University Marburg, Department of Biology, Karl-von-Frisch-Strasse 8, D-35032, Marburg, Germany;* [2]*Philipps-University Marburg, Department of Chemistry, Hans-Meerwein-Strasse, D-35032, Marburg, Germany*

Members of the cryptochrome/photolyase family of proteins use UV-A/blue light either to repair UV-damaged DNA (photolyases) or to trigger various light-dependent processes (cryptochromes). The plant *Arabidopsis thaliana* possesses three cryptochromes two of which (cry1 and cry2) are the conventional ones, and the third one (cry3) belongs to the cryDASH subfamily. A photolyase activity specific for single-stranded DNA was recently identified for members of the cryDASH family[1] thought to be associated with the FAD cofactor as known for the other photolyases. However, their biological role is not clear because cryDASH members, even if strongly overexpressed are not able to confer higher surviving rates of photolyase-deficient *E. coli* strains that have been treated with UV-B and photoreactivating light[1,2]. Both structural and functional[3-6] data demonstrate the presence of a second cofactor (MTHF) in cryDASH members. In photolyases, this second cofactor serves as photoantenna and transfers its excitation energy to the catalytic $FADH^-$ cofactor. In contrast, the role of MTHF in cryDASH members seems to be more complex. We have shown that, in *A. thaliana* cry3, MTHF serves as antenna for photoreduction of FAD under UV-A[4] that is accompanied by so far unidentified photoconversion of MTHF itself.[6] Further analyses suggested that, in addition to UV-A, an electron transport through the conserved Trp triad and/or from $FADH^-$ is required for this phenomenon. Here we present recent data on this important feature as well as some exceptional properties of ssDNA photorepair by *A. thaliana* cry3.

[1]Selby, C.P. and Sancar, A. (2006), *PNAS* **103**(47), 17696-17700. [2]Kleine, T. *et al.* (2003), *Plant J* **35**, 93-103. [3]Saxena, C. *et al.* (2005), *JACS* **127**, 7984-7985. [4]Klar, T. *et al.* (2007), *JMB* **366**(3), 954-964. [5] Huang, Y. *et al.* (2006), *PNAS* **103**(47), 17701-17706. [6]Song, S.-H. *et al.* (2006), *J Photochem Photobiol* B **85**(1), 1-16.

IL519

Light regulates virulence in *Brucella abortus* by a LOV- domain Histidine Kinase protein

Gastón Paris[1], *Diego J. Comerci*[2], *Trevor E. Swartz*[3], *Roberto Bogomolni*[3], *Rodolfo A. Ugalde*[2], *Fernando A. Goldbaum*[1]

[1]*Fundación Instituto Leloir, Buenos Aires, Argentina;* [2]*Instituto de Investigaciones Biotecnológicas, UNSAM CONICET, San Martín, Buenos Aires, Argentina;* [3]*Departament of Chemistry and Biochemistry, University of California, Santa Cruz, California, USA*

Brucella abortus is a facultative intracellular pathogen that causes brucellosis in domestic animals and humans. *Brucella* invades and replicates inside professional and non-professional phagocytes. *B. abortus* contains a LOV-histidine kinase (LOV-HK) protein. LOV domains which belong to the PAS domain superfamily, are the light sensory modules in plant, fungi and algae photoreceptors. LOV domains bind a single molecule of FMN and undergo a self-contained photocycle that is dependent on the presence of a highly conserved cysteine residue. Upon illumination the reactive cysteine forms a covalent bond between the sulfur and C4a carbon of FMN. In order to investigate the *in vivo* function of the *Brucella* LOV-HK a knocked-out null mutant was obtained. Cell infection assays of J774A.1 macrophages showed that LOV-HK knockout strain has an attenuated phenotype as compared with the wild type. A complemented strain expressing the LOV-HK gene in the LOV-HK knockout genetic background was able to rescue the phenotype, with the same replication rate as the wild type. However, a LOV-HK C69A replacement which cannot under go light induced formation of the covalent adduct showed the same infection profile that the LOV-HK knockout strain, indicating that formation of covalent adduct is essential for its biological activity. To determine if LOV-HK functions as photoreceptor during host-pathogen interactions, the infection experiment was performed in light vs. dark conditions. Strikingly, the number of wild type intracellular bacteria recovered from the culture kept in the dark was roughly one order of magnitude less than in the light-treated culture, moreover, no difference between dark and light conditions was detected with the LOV-HK knockout mutant. Activation of LOV-HK protein by light could be part of an adaptive mechanism present in *Brucella* that senses its transition from outside the organism and prepares the bacteria for effective infection of a new mammalian host.

OC520

Ultrafast studies on novel blue-light photoreceptors

Cosimo Bonetti[1], *Tilo Mathes*[2], *Ivo H.M. van Stokkum*[1], *Marie-Louise Groot*[1], *Rienk van Grondelle*[1], *Peter Hegemann*[2], *John T.M. Kennis*[1]

[1]*Section of Biophysics, Department of Physics and Astronomy, Faculty of Sciences, Vrije Universiteit Amsterdam, Amsterdam, Netherlands;* [2]*Institut für Biologie / Experimentelle Biophysik, Humboldt Universität zu Berlin, Berlin, Germany*

Time resolved visible pump, mid-infrared probe and time resolved fluorescence experiments have been performed on a novel class of blue-light photoreceptors, the BLUF (Blue Light sensing Using Flavin) domain family. Time-resolved fluorescence experiments on the Slr1694 BLUF domain from *Synechocystis* revealed four components of flavin adenine dinucleotide (FAD) excited-state decay, with lifetimes of 6 ps, 26 ps, 90 ps, and 340 ps. No kinetic isotope effect on the excited-state lifetime was observed for BLUF domains dissolved in D_2O buffer, indicating that the fluorescence of flavin is quenched by photo-induced electron transfer by a conserved tyrosine residue. In ultrafast transient absorption experiments, excitation at 470 nm triggers the reaction that leads to signalling state formation; the structural changes between flavin molecule and side chain residues inside the protein are followed by monitoring in time the difference absorption over a mid-IR spectral window from 1800-1100 cm^{-1}. We find that the long-lived signalling state is formed on a time scale of 200 ps. In its spectra a down shift of the carbonyl bands appears, indicating a weakening of the C4=O that results from a formation of H-bonds to this group. Beside the carbonyl band, a weakening of C=N stretch vibrations is recorded. Evident contributions from the nearby residues (Y8 and Q50) to the spectra evolution are recorded. Our results support a model where light-induced electron transfer causes a hydrogen-bond rearrangement, whereby the hydrogen bonds from the amino moiety of a highly conserved glutamine to tyrosine and the flavin N5 are broken, followed by a 180° glutamine flip and formation of new hydrogen bonds between the glutamine and FAD.

OC521

Radical formation and autophosphorylation in the light-sensitive domain of the plant cryptochrome from *Chlamydomonas*

Dominik Immeln[1,2], *Ramona Schlesinger*[2], *Joachim Heberle*[1], *Tilman Kottke*[2]

[1]*Department of Chemistry, Biophysical Chemistry, Bielefeld University, 33615 Bielefeld, Germany;* [2]*INB-2: Molecular Biophysics, Research Center Juelich, 52425 Juelich, Germany*

Cryptochromes are sensory blue light receptors mediating responses such as the inhibition of stem elongation, the entrainment of circadian rhythms, and the regulation of gene expression in higher plants[1]. The unicellular green alga *Chlamydomonas reinhardtii* features a single plant cryptochrome, referred to as CPH1[2]. It has been suggested to be ancestral to the higher plant cryptochromes[3].

We have expressed the N-terminal light-sensitive domain (CPH1-PHR) of the protein heterologously in *E. coli* in high yield and purity[4]. The 59 kDa domain bears exclusively flavin adenine dinucleotide in its oxidized state. Blue light induces formation of a neutral flavoprotein radical with maxima of absorption at 540 and 580 nm. Formation of the radical takes place under aerobic conditions and without an external electron donor, indicating that the reaction is of physiological relevance. The radical decays back to the oxidized state in the dark with a time constant of 200s. Binding of ATP strongly stabilizes the radical state by impeding the recovery to the dark state. This effect indicates that the interaction with ATP has a significant impact on cryptochrome function. The light-sensitive domain autophosphorylates upon blue light illumination. This response is detected although the C-terminal domain, the putative signalling domain, is missing. In conclusion, CPH1-PHR represents due to its high homology to higher plant cryptochromes and its evolutionary position a model blue light receptor for investigating two fundamental light-stimulated processes in plant cryptochromes: radical formation and autophosphorylation.

[1]Lin, C. and Shalitin, D. (2003) Annu. Rev. Plant Biol. 54, 469–96; [2]Reisdorph, N. A. and Small, G. D. (2004) Plant Physiol. 134, 1546-1554; [3]Lariguet, P. and Dunand, C. (2005) J. Mol. Evol. 61, 559-569; [4]Immeln, D., Schlesinger, R., Heberle, J., and Kottke, T. (2007) submitted.

P601

UVB-induced DNA damage: high proportion of mutagenic photoproducts in GC-rich genomes

Sabine Matallana Surget[1], Jarah Meador[1,2], Fabien Joux[1], Thierry Douki[2]

[1]Laboratoire d'Océanographie Biologique de Banyuls, CNRS URM7621. Université Paris VI. Avenue Fontaulé. BP44 F-66651 Banyuls-sur-mer Cedex. France, Banyuls sur mer, France; [2]Laboratoire des Lésions des Acides Nucléiques, DRFMC/SCIB UMR E-3 CEA/UJF, Grenoble, France, Grenoble, France

UV radiation is a major environmental mutagen that may affect all forms of life on Earth. A major consequence of exposure to solar UV radiation, and in particular to its energetic UVB portion, is the dimerisation of adjacent pyrimidine bases within DNA. Because of this overwhelming role played by thymine and cytosine, a major effect of the GC content on DNA photobiology is often anticipated. This is particularly relevant to microorganisms where the proportion of AT and GC base pairs vary significantly among species. In the present work, we determined how differences in GC content affected the induction yield and nature of UV-induced photolesions within both cellular and isolated DNA. The two types of UVB-induced DNA lesions, namely cyclobutane dimers and (6-4) photoproducts were quantified individually for each of the pyrimidine dinucleotides by HPLC coupled to mass spectrometry. The determined yields were then correlated to the dinucleotide frequencies reported for the different genomes. The most striking result was the favoured formation of cytosine-containing photoproducts at high GC content. A concomitant decrease in the overall yield of DNA lesions was observed that was mostly accounted for by the decrease in TT intrinsic photoreactivity at high GC content. These observations provide new insight into the basic photochemistry of DNA and also allow a prediction of higher mutagenicity of UV radiation in genomes exhibiting a high GC content. Indeed, mutations at the 3'-end cytosine of bipyrimidine sites are the hallmark of UV light. Similar consideration could also explain hotspots in human genome where GC base pairs are not evenly distributed.

P602

Photoactivated hypericin-mediated DNA damage in HaCaT keratinocytes can be attenuated by vitamin E

Brian Johnston, Nicola J Traynor, Harry Moseley, James Ferguson, Julie A Woods

University of Dundee, Dundee, Scotland

The plant perylenequinone hypericin has potential for use as a photosensitiser in photodynamic therapy (PDT). Microscopy shows that the compound appears to accumulate in the perinuclear region of cultured cells, and can kill by apoptosis or necrosis in a dose-dependent fashion. The combination of hypericin and visible light results in an increase in DNA damage, which can be detected by the single cell gel electrophoresis assay (comet assay). This DNA damage does not correlate with phototoxicity, and may be a “side-effect” of the photodynamic effect. It is detectable even under conditions that are sub-lethal and can be rapidly repaired, although the fidelity of the repair is unknown. PDT is increasingly being used to treat cancerous and benign conditions and although the DNA damage produced seems to be easily handled by the cell, it is important that its nature is understood. Also, if it is not essential for the phototoxic effect then it could be argued that it should be prevented. The pattern of genotoxic damage caused by hypericin-PDT is different from that produced by exogenous hydrogen peroxide (H_2O_2), although both result in mainly strand breaks and oxidative damage. Metal ion chelators are effective in reducing the genotoxic burden of the H_2O_2, but not of hypericin-PDT to any significant level. Histidine was also ineffective, while exacerbating H_2O_2-induced damage. The caspase inhibitor Z-VAD-FMK also did not protect against low dose hypericin-PDT induced DNA fragmentation. In contrast, the major membrane lipid antioxidant, α-tocopherol did confer a degree of protection against hypericin-PDT induced DNA strand breaks, but at high concentrations (50 µM). The comet assay did not reveal the presence of DNA crosslinks. Taken together, these results suggest that one explanation of the DNA damage caused by hypericin-PDT could be endogenously generated distal genotoxins produced by lipid peroxidation of oxidatively stressed membranes.

P603

The crystal and molecular structure of the d(TpA) thymine-adenine photoadduct

R. Jeremy H. Davies[1], John F. Malone[2], Yu Gan[3], Christine J. Cardin[3], Michael P. H. Lee[4], Stephen Neidle[4]

[1]School of Biological Sciences, Medical Biology Centre, Queens University Belfast, Belfast, Northern Ireland, United Kingdom; [2]School of Chemistry, Queens University Belfast, Belfast, Northern Ireland, UK; [3]School of Chemistry, University of Reading, Reading, UK; [4]The School of Pharmacy, University of London, London, UK

A high resolution crystal structure has been determined for the intramolecular thymine-adenine photoadduct (TA*) that is produced by direct UV excitation of the dinucleoside monophosphate d(TpA). Although its quantum yield for formation in DNA is much lower than for pyrimidine photodimerization, TA* has been implicated as a mutagenic lesion in UV-irradiated bacterial and human cells.

As originally predicted from heteronuclear NMR analysis, TA* contains a tricyclic ring system in which a central 8-membered diazacyclooctatriene ring is fused to the pyrimidine ring of thymine and the imidazole ring of adenine. Within the crystal, it exists as a zwitterion with a protonated amidine function in the 8-membered ring neutralising the charge of the internucleotide phosphate monoanion. The photoproduct has an asymmetric structure with distorted backbone geometry and glycosidic angles that differ by more than 80º. The pucker of the 3'-deoxyribose moiety is C2'-*endo* while that of the 5'-deoxyribose is in the C3'-*endo* range.

The absolute configuration at the original thymine C(5) and C(6) atoms in TA* is defined as 5*S*,6*R*. This stereochemistry is compatible with TA* being generated by valence isomerization of a precursor *cis-syn* cyclobutane photoadduct whose formation would be favoured by the stacked conformation of adjacent T and A bases in B-form DNA. It is likely therefore that the primary photoreaction leading to TA* is mechanistically analogous to pyrimidine dimerization despite having a much reduced quantum efficiency.

P604

Radiation-induced clustered DNA damage

Noureddine Belmadoui, Peggy Regulus, Jean-Luc Ravanat, Didier Gasparutto, Jean Cadet

Laboratoire Lésions des Acides Nucléiques, DRFMC-SCIB-E n. 3 CEA-UJF, CEA-Grenoble, Grenoble, France

Cellular components, in particular DNA, are targets to a large number of endogenous or exogenous genotoxic agents. Among them, Reactive Oxygen Species (ROS), produced under oxidative stress conditions, UV and γ irradiations, may damage DNA. The resulting generated lesions include base modifications, strand breaks, sugar alterations and DNA-protein cross-links. The cytosine modification, so-called dCyd341 is one of the main radiation-induced DNA modifications. The formation of dCyd341 is explained in terms of reaction of an oxidized sugar residue with a vicinal cytosine base. In a recent work the structure was confirmed and a mechanism of formation for dCyd341 has been proposed. It is interesting to understand the undergoing reactions initiated by C4'-hydrogen abstraction. In particular, the use of a photolabile precursor of C4' radical would be of interest to determine the yield of the reactive aldehyde involved in the cytosine adduct formation and to gain insights into mechanistic aspects of the reaction. In

addition such an approach is likely to allow determining the efficiency of the reaction of the aldehyde with proximal cytosine according to their relative position in double-stranded DNA. The reaction of the sugar aldehyde, with a vicinal cytosine, may induce, if the base is located on the complementary strand, the formation of an inter-strand DNA cross-link. The current approach to measure dCyd341 that requires complete DNA digestion cannot provides information on the nature of the inter- or/and intra-strand cross-links that are formed. It may be added that repair studies have revealed the ability for the cells to excise the lesion. Identification of the repair system(s) involved in the removal of the clustered damage from DNA constitutes another challenging issue.

P605

The GTPase RhoB, a UVB-responsive protein, regulates p53-independent apoptotic pathways in human normal keratinocytes

Bruno Canguilhem[1], *Anne Pradines*[1], *Marie Charvéron*[2], *Gilles Favre*[1]

[1]*INSERM U563, Toulouse, France;* [2]*Institut de Recherche Pierre Fabre, Toulouse, France*

Ultraviolet irradiation generates many deleterious effects on skin tissue leading to fast adaptive cell response. Dysregulation of this response promotes premature photo-aging and skin cancer. In addition to genetic alterations, inappropriate growth, differentiation and/or apoptotic response play key roles in photocarcinogenic process. Previous studies from our laboratory have shown that the short-lived small GTPase RhoB was strongly up-regulated after UVB exposure in HaCat keratinocytes cell line. Using the same cellular model we have also shown that specific inhibition of RhoB induction increased the UVB apoptotic response through its involvement in the EGF receptor pathway. These results put on light RhoB as a protective determinant in UVB cellular response and suggest its potential role in photocarcinogenesis.

In the immortalized HaCat cells, the protein p53 is mutated and these cells are more sensitive to apoptosis than normal keratinocytes. Thus, in the presented work we address the questions of the regulation of RhoB and of the link between this protein and the p53-regulated apoptotic pathways in normal models (i.e. human skin and epidermal keratinocytes primary cultures). We first have studied the localization of RhoB protein in human skin and its regulation in response to UVB. Then we have compared the basal expression level of RhoB between several donors and linked it to a polymorphic sequence of its promoter. Finally, we have analyzed the involvement of RhoB in the UVB-induced apoptotic response. Altogether these results lead us to confirm the previous results obtained in the HaCat model and demonstrate that RhoB regulate UVB-response in a p53-independent way.

P606

Wavelength dependence of cell cycle responses in human melanocytes and melanoma cells following exposure to ultraviolet radiation

Christine Kowalczuk, *Marisa Priestner, Rick Saunders, Simon Bouffler*

Health Protection Agency, Chilton, Oxfordshire, United Kingdom

Animal studies have raised the possibility that long wavelength UVA may be more effective at inducing melanoma than DNA damage action spectra would predict. This study has examined the wavelength dependence of the effects of UVR on the cell cycle response of human melanocytes and melanoma cells. Primary human melanocytes, isolated from juvenile foreskin tissue, and G361 human melanoma cells were exposed to 254 nm germicidal UVC radiation (0 - 30 J m^{-2}), 311 nm UVB radiation (0 – 3 kJ m^{-2}), or broadband UVA radiation (Sellamed 4000, maximum output between 350 - 450 nm) (0 – 1.2 MJ m^{-2}). Flow cytometry was used to monitor cell cycle distributions for up to one week post-irradiation and western blot analyses of G1/S checkpoint related cell cycle proteins (p14, p16, p21, p27 and p53) are in progress. Cell cycle distributions showed a sustained, dose-dependent G2/M block following exposure of melanoma cells with all wavelengths and a slowing of transit through S phase following UVA irradiation. There was no apparent block to G1 cells entering S phase at any wavelength. Melanocytes, on the other hand, showed a marked G1 arrest, particularly following UVA irradiation. These results show that G361 malignant melanoma cells have lost the ability to regulate the cell cycle at the G1/S checkpoint. This is supported by preliminary western blot analyses showing a lack of p16 expression in the melanoma cells. UVA nevertheless induced strong cell cycle delays in both cell types, indicating that UVA exposure can significantly affect genome metabolism. Further results of the protein expression studies will also be presented.

P607

Role of uv-induced oxidative stress in the regulation of the GTPase RhoB

Bruno Canguilhem[1], *Sarah Blaquière*[2], *Hélène Hernandez-Pigeon*[2], *Anne Pradines*[1], *Marie Charvéron*[2], *Gilles Favre*[1]

[1]*INSERM U563, Toulouse, France;* [2]*Institut de recherche Pierre Fabre, Toulouse, France*

Our previous studies have shown that the small GTPase RhoB regulates the balance between apoptotic and survival response of human keratinocytes to UV radiation and for this reason RhoB account for a new potential mediator of photocarcinogenic process. This protein is strongly up-regulated after exposure of keratinocytes to UV. We previously have shown that UVB induce a fast activation of RhoB followed by a long-lasting increase of its expression. The presented study is designed to determine the mechanisms responsible for this regulation. Literature strongly suggest that ROS may be involved in the regulation of activation and expression of the Rho GTPases. Skin exposure to UV radiation generates ROS in excessive quantities that quickly overwhelm tissue antioxidants. Uncontrolled release of ROS is involved in the pathogenesis of a number of human skin disorders including cutaneous carcinogenesis. Here we report that RhoB basal expression is mediated by ROS. In response to UV radiation, we show that ROS participate to RhoB regulation. Pre-treatment with the antioxidant N-acetyl-L-cysteine (NAC) completely inhibits RhoB protein induction after UVA exposure. After UVB exposure, RhoB protein increase is only reduced, underlining a regulation most likely due to DNA damage, mostly induced by the UVB part of the UV spectrum. Antioxidants do not modified the RhoB mRNA up-regulation, highlighting that only protein stability or degradation is regulated by ROS. Moreover, we report the involvement of ROS in the regulation of the UV-induced activation of RhoB. This study provides new insights about the mechanisms responsible for the regulation of RhoB and offers new targets for the investigation of the role of RhoB in the UV-response.

P608

Molecular responses of normal human caucasian melanocytes in culture exposed to simulated solar UV: could melanin and its precursors behave as endogenous photosensitizers?

Laurent Marrot, *Jean-Philippe Belaidi, Christophe Jones, Philippe Perez, Jean-Roch Meunier*

Phototoxicity Unit, Safety Research Dpt, L'OREAL, Aulnay-sous-Bois, France

Melanocytes have a central role in the response of skin to sunlight exposure. They are directly involved in UV-induced pigmentation as a defence mechanism. However, their alteration can lead to melanoma, a process where the role of sun overexposure is highly probable. The aim of this work was to analyze the behaviour of melanocytes from fair skin under irradiation mimicking environmental sunlight in terms of spectral power distribution. To

do this, normal human Caucasian melanocytes in culture were exposed to UV radiation from a solar simulator (SSUV: 300-400 nm). Even at relatively high doses (12 kJ/m^2 UVB and 110 kJ/m^2 UVA), cell death was limited. Moreover, p53 accumulation was three times lower in melanocytes than in unpigmented cells such as fibroblasts after SSUV exposure. However, an important fraction of melanocyte population was arrested in G2/M phase, and this correlated well with a high induction level of the gene *GADD45*, *4* hours post-exposure. Among the genes involved in DNA repair, gene *XPC* was the most inducible since its expression increased more than two fold 15 hours, whereas expression of *P48*, also involved in the nucleotide excision repair pathway, was only slightly increased. In addition, an early induction of Heme Oxygenase 1 (*HO1*) gene, a typical response to oxidative stress, was observed for the first time in melanocytes. Interestingly, this induction remained significant when melanocytes were exposed to UVA radiation only (320-400 nm) and stimulation of melanogenesis prior to irradiation further increased *HO1* induction. Such a behavior confirmed previous results showing that photoinduced DNA breakage by UVA (detected using the comet assay) was also enhanced when melanogenesis was triggered. This work provides new data about the stress reponse of human melanocytes exposed to UV and underlines the probable involvement of sunlight in melanoma initiation.

P609
Wavelength dependency of ultraviolet radiation induced skin infiltration of neutrophils

Peter L. Lee[1], *Huib van Weelden*[1], *Piet L.B. Bruijnzeel*[2]
[1]*University Medical Center Utrecht, Utrecht, the Netherlands;* [2]*Organon Biosciences B.V., Oss, The Netherlands*

Exposure of the skin to ultraviolet radiation (UV) may induce infiltration of neutrophils. This finding raised the question whether the infiltration of neutrophils is wavelength dependent. To answer this question the infiltration of neutrophils in the skin was investigated after exposing skin of healthy individuals to a single erythemal dose of different UV sources: solar simulated radiation (SSR), broadband UVB (BB-UVB), narrowband UVB (NB-UVB) or UVA1. Biopsies were collected at 6 and 24 hours after exposure. At 6 hours after irradiation to 2 MED both SSR and NB-UVB were equally potent in mobilising significant numbers of neutrophils in the dermis. At this point of time BB-UVB and UVA1 hardly induced infiltration of neutrophils in the dermis. At 24 hours after exposure to SSR or NB-UVB the number of neutrophils had increased significantly and in some cases neutrophils were even present in the epidermis. At that time point exposure to BB-UVB also induced dermal influx of neutrophils but considerably less than SSR or NB-UVB. UVA1 was even less potent than BB-UVB. The similar response in neutrophil influx seen with the SSR and NB-UVB sources may be due to the fact that both sources have in common that they emit predominantly longer wavelengths within the UVB range. Therefore longwave UVB most likely contributes to the infiltration of neutrophils.

P610
Limitations of UV-A1 high dose therapy

Helmut Piazena
Klinik für Psychiatrie und Psychotherapie der Charité, Berlin, Germany

Irradiations with high doses of long-wave ultraviolet (UV-A1, 340 nm – 400 nm) are used for treatment of atopic dermatitis and other skin diseases. Devices for UV-A1 high dose therapy have to render skin exposures with (unweighted) doses in the UV-A1 range of about 130 J cm^{-2} within 30 min and without exceeding the Minimal Erythema Dose (1 MED = 250 J m^{-2}). In contrast, limitations of (unweighted) maximum doses of between about 61 J cm^{-2} and about 91 J cm^{-2} due to exceeding the MED were calculated for all available types of devices intended for UV-A1 high dose therapy. The calculations were performed according to $H_{UVA1,max} = E_{UVA1} \cdot T_{er}$ on the basis of spectral irradiance measurements which were used to determine both (unweighted) irradiance in the UV-A1 range (E_{UVA1}), erythema effective irradiance (E_{er}) and the exposure time to get 1 MED (T_{er} = 250 J m^{-2} / E_{er}). This result was supported by both reported UV erythema formation in the skin of patients treated with doses below 130 J cm^{-2} by different UV-A1 high dose devices as well as by observed maximum UV-A1 doses between about 40 J m^{-2} and about 84 J m^{-2} in the skin of volunteers with photo-types I – III which were inline with calculated maximum doses limited by erythema formation. Based on analysis of spectral filtering upon the ratio between unweigthed irradiance and erythema effective irradiance, the cut-off wavelength of a rectangular spectrum has to exceed about 375 nm to allow UV doses of 130 J cm^{-2} without exceeding the MED whereas cut-off wavelengths between 378 nm and 383 nm were calculated for the UV-A1 devices evaluated in dependence on the used lamp type. In contrast, the devices were filtered at wavelengths of about 340 nm. Furthermore, spectral filtering in needed dimensions would decrease the remaining irradiances of the evaluated devices between about 51 % and about 81 % resulting in corresponding extension of exposure times up to several hours to get the therapeutic dose. However, the use of the *Bunsen-Roscoe* law of proportionality to compensate decreases of irradiance by equivalent increases of exposure time is only justified in the absence of secondarily stimulated effects in the tissue such as repair or pigmentation which could cause not only an additional extension of exposure time, but also the prevention of the therapeutic effect at all. It was concluded that the defined conditions to perform UV-A1 high dose therapy are unattainably in principle if devices are used which were equipped with currently available UV sources. Thus, it is recommended to take critical review of the dose-effect-relationships reported in basic publications to establish UV-A1 high dose therapy which obviously were used to define photobiologically unfounded conditions. Furthermore, it would seem advisable to replace the recommended dose for UV-A1 high dose therapy by a more realistic value.

P611
The effect of the short wavelength ultraviolet radiation. An extension of biological dosimetry to the UV-C range

Berces A., Kovacs G., Ronto G.
Semmelweis University, Dept. Biophyisics & Radiation Biology, Budapest, Hungary

Polycrystalline uracil thin layer can be used as biological dosimeter for assessing exposure to UV radiation. The dimerization and reversion efficiency of the ultraviolet radiation in the UV-B and the UV-C range were quantified on polycrystalline uracil thin layers irradiated with quasi-monochromatic radiation using interference filters of 10 nm bandwidth. The dimer formation and monomerization (reversion) dose-effect relations were determined by optical spectroscopy. The decrease of the OD value of the uracil thin layer at 288 nm was taken as a measure of the dimer formation, while the increase of the OD of a completely irradiated (until reaching the saturation level) uracil layer was taken as the sign of the monomerization. The two processes in the UV-B and the UV-C range take place simultaneously, the individual characterization of the dimerization efficiency was performed from the initial slope of the dimerization dose-effect function and an action spectrum for dimerization was constructed in the UV-C range too. The reversion efficiency was found to be practically the same with all of the investigated wavelengths: 200 nm, 210 nm 220 nm, 230 nm, 240 nm The possible biological relevance of the reversion of dimers are discussed.

P612

Inactivation of bacteria in platelet concentrates by UVC irradiation

Harald Mohr, Ute Gravemann, Anette Bayer, Thomas H. Müller

Blood Center of the German Red Cross Chapters of NSTOB, Springe, Germany

Introduction: Short-wave ultraviolet light (UVC) is germicidal, but low UV permeability of turbid suspensions hampers its use for sterilizing platelet concentrates (PC). A simple method was found which overcomes this limitation.

Methods: PC in storage medium SSP+ (volume approx. 350 mL, platelet concentration approx. 10^9/mL, plasma content approx. 30 %) were prepared from pools of 5 buffy coats. In experiments in which inactivation kinetics were determined, the PC were spiked with 10^5-10^6 colony forming units (CFU)/mL of different bacteria species. In others directed to the sterilization of PC, spiking concentration was approx. 100 CFU/mL. Unspiked PC were used to investigate the influence of UVC on platelet parameters. UV transparent plastic bags containing PC or PC-aliquots were either clamped between quartz plates or remained free. Bags were irradiated on both sides with UVC light at 0.1 – 0.6 J/cm^2 under agitation (up to 110 rpm). The sterility of PC spiked with lower numbers of bacteria was tested the day after UVC treatment and after storage at 22°C for 6 days.

Results: Bacteria inactivation was strongly enhanced when the PC were agitated during irradiation without clamping. UVC-light at 0.4-0.5 J/cm^2 reduced the titers of all bacteria species tested by approx. 5-6 $\log_{10}$. PC spiked with approx. 100 CFU/mL of bacteria were reproducibly sterilized at the same dosage. Most PC properties remained almost unchanged after treatment. The storage stability of the treated PC for up to 6 days after treatment (8 days after blood donation) was, however, maintained.

Conclusions: UVC-light under strong agitation effectively inactivates bacteria in PC. Under optimized conditions the influence on in vitro platelet parameters is marginal.

P613

Photo-induced crosslinking of isolated calf-lens proteins and crystallin fractions sensitized by advanced glycation endproducts

Denis Fuentealba, Eduardo Silva

Laboratorio de Química Biológica, Departamento de Química Física, Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile

Advanced glycation endproducts (AGEs) have been a subject of permanent interest through the last decade since they are related to many chronic diseases. Along with the multiple consequences of AGEs formation in vivo, is their photosensitizing activity, which is of special relevance in tissues that are exposed to light, particularly the eye lens, where they are thought to be related to cataract development. Within the lens, AGEs act as endogenous sensitizers generating reactive oxygen species upon irradiation with UVA light as it has been demonstrated in vitro. Nonetheless, the actual potential of damaging lens proteins has not been addressed properly. More over, the real conditions of the lens, which is a poorly oxygenated tissue, had not been considered. In a previous investigation, we studied the photosensitizing activity of lysine derived AGEs upon irradiation with UVA-visible light in the presence of important targets within the lens such as tryptophan residues, protective enzymes and ascorbate. In the present work, we addressed the matter of the eye lens photodamage in terms of protein crosslinking, sensitized by in vitro generated AGEs. The influence of the oxygen concentration in the photosensitizing mechanism is further discussed. AGEs were prepared by incubating a mixture of *N*α-acetylated lysine and arginine with glucose, and characterized by absorption and emission spectroscopy. Isolated calf-lens proteins were irradiated with UVA-visible light in the presence of AGEs under 5% oxygen pressure. SDS-PAGE analysis of the samples revealed the formation of covalently crosslinked products. The observed crosslinking was partially inhibited by the presence of ferricyanide, which is consistent with the occurrence of type I photosensitizing mechanism. More over, the protein crosslinking was not affected by the increase in oxygen concentration, indicating a preference of type I interaction over type II sensitizing mechanism. The behavior of isolated α-, β_H- and β_L-crystallins was also examined. All of the results are consistent with AGEs acting as type I sensitizers and providing experimental support of a radical-mediated protein crosslinking. The results show the potential of AGEs photosensitizing activity of inducing eye lens protein crosslinking, which could contribute to the photodamage of this tissue.

This work was supported by FONDECYT (Grant 1050965) and CONICYT (Grant AT-23070007). D. Fuentealba thanks CONICYT for a Doctoral fellowship (21060405). Special thanks to A. Matus who extracted and purificated the crystallins used in this study.

P614

Singlet oxygen-mediated tryptophan oxidation: characterization of potential markers of protein oxidation

Michelle Gracanin, David I. Pattison, Michael J. Davies

The Heart Research Institute, Sydney, NSW 2050, Australia

Proteins are major biological targets for oxidative damage within cells due to their high abundance and their rapid rates of reaction with both a wide range of free radicals and a number of excited state species including singlet oxygen. Exposure of some free amino acids, peptides and proteins to singlet oxygen has been shown to generate peroxide species in high yield, with these species formed predominately on Tyr, Trp, and His residues. Protein peroxides have also been detected on proteins within intact cells on exposure to a photosensitizer and light. The chemical structures of some of these materials, and their breakdown products, have been elucidated, particularly with the free amino acids, but much less is known about the nature of the peroxides and their decomposition products on intact proteins. In the study reported here we have characterised the structures of Trp-derived peroxides and breakdown products on the free amino acid, in peptides and on proteins using liquid chromatography-mass spectroscopy (LC-MS). Illumination of free Trp with visible light in the presence of the sensitizer Rose Bengal gives rise to 7 major products that have been characterized in detail. The products include two isomeric hydroperoxides, two alcohols, two diols and N-formylkynurenine, with these materials consistent with photo-oxidation occurring via the formation and subsequent reactions of singlet oxygen. The hydroperoxides are readily decomposed at elevated temperatures and in the presence of reductants, to give increased amounts of the alcohol products. These materials are acid-sensitive. In order to examine whether some of these species can be used as markers of damage induced by singlet oxygen we have used pronase to digest the photo-oxidised proteins to free amino acids. Under the conditions employed, some of these materials are sufficiently stable to be quantified post-hydrolysis by LC-MS, and hence can be used as markers of singlet oxygen generation and damage to proteins. This approach may allow the quantification of protein modification in intact cells arising from singlet oxygen formation.

P615

Effects of low power laser on plasmatic glycerol: an experimental study in rats

Halina Camargo Senhorinho, Gerson Linck Bichinho, Munir Antônio Gariba, Percy Nohama

Pontifical Catholic University of Paraná - PUCPR, Curitiba, Paraná, Brazil

Introduction: Low power lasers have been used as therapeutic devices for over 35 years. When interacting with biological tissues, they generate photobiological processes referent to modulation and,

consequently, macroscopic effects. Therefore, one of the applications of lasers as a therapeutic modality aims at reducing body contour deformities, though little is known about the effects of lasers on fatty tissue. In view of this, the aim of this first study consisted in evaluating the influence of a low power laser (In-Ga-Al-P) on plasmatic glycerol in rats.
Methods: This study was approved by the Research Ethics Committee of the Pontifical Catholic University of Paraná – PUCPR. The sample consisted of 20 adult female Rattus norvergicus albinos from Wistar strain, with six months of life, randomized in four groups: L1, L2, L3 and L4. All groups were submitted to tricotomy of the dorsal thoracic region and the first three groups were exposed to the In-Ga-Al-P Endophoton® laser, model LLT 0107 from KLD Biosystems, with 0.035 cm^2 of transmission area, in continuous mode, with 20 mW, 660 nm wavelength and fluency of 2 J/cm^2 for L1, 8 J/cm^2 for L2 and 16 J/cm^2 for L3 groups. L4 was the control group. The groups were submitted to the protocols three times a week, for three weeks. The evaluation consisted of the quantification of plasmatic glycerol in two moments: prior and after the last irradiation with the laser. For this analysis, a specific enzymatic kit, from Laborclin laboratory products LTDA, was used.
Results: The mean difference between the values of plasmatic glycerol prior and after laser application were: 0.0075 ±0.0050 mg/dl, -0.0047 ±0.0064 mg/dl and -0.0009± 0.0080 mg/dl for L1, L2 and L3, respectively. From the Wilcoxon test, statistical significance was obtained only for L1 ($p<0.043$).
Conclusion: From these results, it can be concluded that the application of the low power laser (InGaAlP) with energy density of 2 J/cm^2, in the conditions proposed, increases the concentrations of plasmatic glycerol, suggesting a lipolysis stimulus.

P616

Visible-light-induced respiratory rate increase in cells: searching for a plausible photoacceptor

A. Remedi[1], L. di Salvo[1], G. Cercignani[1,2], S. Lucia[1], G. Colombetti[1]
[1]Istituto di BioFisica, CNR, Pisa, Italy; [2]Dipartimento di Biologia, Università di Pisa, Pisa, Italy

Recently some research groups have investigated the effects of visible-to-near-infrared (Vis-NIR) irradiation on several kind of tissues, reporting the fast cell recoveries from different kind of stresses.[1-3] Along the same research theme, the NASA developed some LED prototypes for phototherapeutic skin irradiation.[4] The hypothesis has been supported that these effects are mediated by the mitochondrial enzyme cytochrome *c* oxidase (CcOX).[5] Our goal is to quantify the effects of Vis-NIR irradiation and assess whether they could be shown at any of the spectral regions where CcOX absorbs, both on a purified CcOX preparation and on living cells. We measure, through a Clark electrode under conditions of steady-state reduction of cytochrome *c*, the O_2 consumption rate *during* Vis-NIR irradiation on the *in vitro* activity of the bovine purified CcOX and on the *in vivo* activity of the same enzyme in cultured cells. Under our experimental conditions, the activity of purified CcOX is unaffected by irradiation with any frequency of Vis-NIR light, whereas on cultured mammalian cells and on *Tetrahymena thermophila* cells we measure a sudden increase in O_2 consumption rate during Visible irradiation. Under the same conditions, NIR light has no effect on the same metabolic parameter. It is also very interesting that Visible light induces a strong effect on aerobically grown *Saccharomyces cerevisiae* cells, but has no relevant effect on the anaerobically grown cells. The dose-response curves obtained on *T. thermophila* show that most of this effect is due to the blue component (420±30 nm), while red light (> 600 nm) produces a lower effect than blue light, consistent with the absorption spectrum of CcOX. These results seem to confirm that CcOX is the specific photoacceptor involved in these phenomena, and suggest an underlying mechanism of photobiomodulation of the respiratory rate in eukaryotic cells.

[1]Karu, T.I. (1999) *J Photochem Photobiol B:Biol* **49**:1-17; [2]Wong-Riley, M.T.T. *et al.* (2005) *J. Biol. Chem.* **280**:4761–4767; [3]Seme, M.T. *et al.* (2001) *Invest. Ophthalm. Vis. Sci.* **42**:834–841; [4]Whelan, H.T. *et al.* (2003) *J Clin Laser Med & Surgery* **21**:67-74; [5]Karu, T.I. *et al.* (2004) *Photochem Photobiol Sci* **3**:211–216.

P617

Refinement and use of an *in vitro* model for the measurement of the vitamin D production capacity of sunlight

Alex Russell McKinley[1], Michael G. Kimlin[1], Michael R. Moore[2]
[1]Queensland University of Technology, Brisbane, Queensland, Australia; [2]National Research Centre for Environmental Toxicology, Brisbane, Queensland, Australia

The measurement of the vitamin D production capacity of sunlight occurs infrequently, and when measured is generally extrapolated from erythemal UV data. The action spectra for the production of erythema and of vitamin D are different, and it is less than ideal to attempt to quantify vitamin D production from the measurement of erythemal UV. As many studies are finding that a large number of people from all walks of life have insufficient levels of vitamin D, it would be very beneficial to have a trusted tool to measure the sun's ability to produce vitamin D. This project refines a method for the in-vitro measurement of vitamin D production and subsequent sample analysis by way of HPLC (High Performance Liquid Chromatography). An in-vitro model making use of 7-dehydrocholesterol mixed in ethanol was used to produce pre-vitamin D when exposed to simulated solar UV radiation in a laboratory setting. This model was shown to provide a high quality dose response relationship. HPLC analysis of exposed samples resulted in rapid and highly accurate sample analysis. The in-vitro model was then put to use in a study measuring vitamin D production capacity of sunlight in the urban canyon of Brisbane, Australia. Methodology and HPLC analysis procedures along with the results of the urban canyon study are discussed.

P618

Scattered UV radiation, shade structures and vitamin D

David J Turnbull, Alfio V Parisi
Centre for Rural and Remote Area Health, University of Southern Queensland, Toowoomba, Queensland, Australia

The health effects of solar UV radiation vary significantly, from assisting calcium absorption in humans due to the initiation of the synthesis of vitamin D_3 to the severe degradation of body tissue. The good effects are relatively few, but they are essential to the well being of humans. It is well known that exposure to small amounts of UVB (280 to 315 nm) radiation are beneficial for the human body and important in the production of vitamin D_3, whereas excessive exposure to UVB and UVA (315 to 400 nm) is known to cause skin cancer, DNA damage, immune suppression, erythema and sun-related eye disorders. It is estimated that approximately 90-95% of our vitamin D_3 requirement comes from exposure to the sun. Studies on the levels of UV measured in the shade of different shade environments have shown that the ratios of UVB to UVA in the shade are significantly different to that in full sun. The ratio of UVB to UVA is higher in the shade than in full sun. These differences are due to the phenomena of Rayleigh scattering ($\propto\lambda^{-4}$) and Mie scattering ($\propto\lambda^{-1}$) in the atmosphere, which causes greater scattering at the shorter UVB wavelengths compared to that at the longer wavelength UVA. Therefore, certain shaded environments may hold the effective wavelengths needed for vitamin D_3 production in the body, but not the high levels of UVA experienced in full sun. At certain latitudes and solar zenith angles, unprotected exposure for short periods to the UVB under shade structures may be the best course of action as it will contribute more toward vitamin D production than toward erythema

compared to exposing the body to full sun UV due to the reduced relative component of the UVA in the shade.

P619

First experiences in using broadband instruments and electronic personal dosimeters designed for erythemally effective UV radiation to measure the Vitamin D effective UV radiation

Alois W. Schmalwieser[1], Peter Koepke[2]

[1]University of Veterinary Medicine, Institute of Medical Physics and Biostatistics, Vienna, Austria; [2]Meteorological Institute, Department of Physics, L.-M.-University Munich, Munich, Germany

A variety of broadband instruments are in use to measure the erythemally effective UV radiation. In many countries UV monitoring networks were established and equipped with such broadband devices. These broadband instruments are generally mounted horizontally but first approaches were made to measure the UV sky distribution operationally too. Nowadays also electronic personal dosimeters (high temporal resolution, small size and low weight) of satisfying quality are available and used in field studies.

In recent years the interest in Vitamin D photosynthesis initiated by solar radiation has risen rapidly. However less data are available - especially continuous recordings. It is therefore of special interest if the measurements of the already installed devices can be used for measuring the Vitamin D effective UV radiation.

In our study we have proven the possibility to use devices which were designed originally for erythemally effective radiation to measure the Vitamin D effective UV radiation. As for the erythemally effective radiation conversion procedures have to be applied to convert the measurements to biologically effective quantities. Beside on the physical properties of the instruments, conversion depends also on temporal variable parameters like solar height and total ozone. The action spectrum of Vitamin D photosynthesis differs especially in the UVA from the action spectrum of the human erythema. With that, a higher sensitivity to solar height and to the total ozone content and its uncertainties is found. Relative uncertainties of measurements increase for low solar heights and high total ozone values. Low solar elevation and high total ozone values occur on the Northern hemisphere from October to March. This is also the critical period in respect to the Vitamin D level in people. High standards in quality control and quality assurance are necessary to enable proper measurements of the Vitamin D effective UR radiation.

P620

Utilising polyphenylene oxide for long term solar UVA dosimetry

David John Turnbull[1], Peter W Schouten[2]

[1]Centre for Rural and Remote Area Health, University of Southern Queensland, Toowoomba, Queensland, Australia; [2]University of Southern Queensland, Toowoomba, Queensland, Australia

Exposure to UV radiation is known to be a causative factor in the induction of skin cancers and other sun-related disorders. Most acute responses of humans to UV exposure occur as a result of UVB (280 to 315 nm) exposures, as these wavelengths are highly sensitive in creating a human biological response. However, this does not mean that UVA radiation has no impact on human UV exposures and health. UVA can cause erythema in human skin, yet, the exposures required to create such a response is much larger than UVB radiation. UVA radiation penetrates much deeper into human skin tissue than UVB, resulting in impacts that are not as acute, taking many years to manifest. Past research has shown that UVA (315 to 400 nm) plays a significant role in human skin carcinogenesis. Studies have also shown that UVA plays an important role in skin damage, immune suppression, DNA damage, photoageing and wrinkling. Researchers at the University of Southern Queensland have developed a personal UV dosimeter that can quantitatively assess long term solar UVA exposures. The chemical polyphenylene oxide, cast in thin film form and which is responsive to both the UVA and UVB part of the spectrum was used and filtered with mylar. This combined system responded to the UVA wavelengths only and underwent a change in optical absorbance as a result of UVA exposure. Preliminary results indicate that this UVA dosimeter saturates reasonably slowly when exposed to sunlight and can measure exposures of more than 20 MJm^{-2} of solar UVA radiation with an uncertainty level of no more than ± 5%.

P621

Costa del Salford: emulating summer UV exposure in north-west England

Richard Kift, Lucy Bunhill, Donald Allan, Marie Durkin, Jacqueline Berry, Lesley E. Rhodes, Ann R. Webb

University of Manchester, Manchester, United Kingdom

As part of the CRUK project investigating the level of Vitamin D in the general population an intervention study was performed in the winter of 2007 to try and mimic summer UV exposure and quantify its effect on vitamin D levels. The lamps chosen for the sunbed were the best spectral match available for Manchester sunlight. The exposure time for the volunteers was chosen to provide the equivalent of casual lunchtime exposure in the summer, whilst remaining well below the MED levels for any volunteer. Also clothing for each volunteer was chosen to maintain the same skin exposure for all volunteers and to mimic summer dress styles. The sunbed was monitored spatially and spectrally throughout the study and exposure times adjusted to maintain constant effective UV doses throughout the study period. The spectral irradiance of the sunbed, and sunlight, were measured with a double monochromator spectroradiometer and the sunbed found to have an erythemally effective irradiance 2-3 times as high as a cloudless, mid-summer midday solar spectrum. We were thus able to provide a summer sunlight exposure regime in January, when it is not possible to produce vitamin D from natural sunlight.

P622

Clear sky UV simulations in the 21st century based on CCM predictions

Kleareti Tourpali[1], Alkiviadis Bais[1], Andreas Kazantzidis[1], Neal Butchart[2], Christoph Brühl[3], Martin Chipperfield[4], Martin Dameris[5], M.A. Giorgetta[6], U. Langematz[7], Eva Mancini[8], E. Manzini[9], Gianni Pitari[8], E. Rozanov[10]

[1]Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Met Office Climate Research Division, Exeter, UK; [3]Max Planck Institut für Chemie, Mainz, Germany; [4]Institute for Atmospheric Science, University of Leeds, Leeds, UK; [5]DLR, Institut für Physik der Atmosphäre, Oberpfaffenhofen, Germany; [6]Max Planck Institut für Meteorologie, Hamburg, Germany; [7]Freie Universität Berlin, Berlin, Germany; [8]Università L'Aquila, Dipartimento di Fisica, L'Aquila, Italy; [9]Istituto Nazionale di Geofisica e Vulcanologia, Bologna, Italy; [10]Institute for Atmospheric and Climate Science ETHZ and Physical-Meteorological Observatory,World Radiation Center, Davos, Switzerland

Future solar UV radiation levels will depend on the evolution of various factors, known to influence the propagation of UV radiation in the atmosphere. Some of these factors, such as ozone, clouds and surface reflectivity are included in coupled Climate Chemistry Models (CCM) output, whereas the prediction of future aerosol levels and their optical characteristics, important for UV radiation, is presently not feasible. Under clear skies, the most important factor for UV-B radiation is stratospheric ozone, followed by tropospheric ozone and aerosols.

In this preliminary study we have used monthly mean total ozone (TOZ) as provided by CCMs taking part in SOUT-O3 Activity 1. TOZ data are an outcome of simulations run under the REF2 and SCN2 scenarios, consistent with the reference simulations proposed by CCMVal. TOZ is then used as input to a radiative transfer model (LibRadTran) for the simulation of the corresponding future UV irradiance levels, presented here as time series of monthly erythemal irradiance received at the surface during local noon, with a time span following the CCM output.

P623

Abnormal IL-4 and IL-12p70 secretion in stimulated peripheral blood mononuclear cells (PBMC) from patients with Polymorphic Light Eruption (PLE) is unaffected by TL-01 phototherapy

Alison Blackburn[1], Sandra M. Winhoven[1], Paul Balmer[2], Margaret Brownrigg[1], Lesley E. Rhodes[1], Neil K. Gibbs[1]
[1]Dermatological Sciences, University of Manchester, Manchester, UK; [2]HPA Vaccine Evaluation Department, Manchester Royal Infirmary, Manchester, UK

The pathogenesis of Polymorphic Light Eruption (PLE) may involve an abnormal T-cell response to a 'photoallergen' generated following skin exposure to solar ultraviolet radiation (UVR). To examine whether this putative 'photo-autoimmunity' is associated with a systemic Th1/Th2 imbalance we have investigated the cytokine secretion profile of phytohaemagglutinin (PHA) stimulated PBMC from normal (n=10) and PLE subjects before (n=10) and after (n=7) a successful course of TL-01 'photohardening' therapy (Winhoven *et al*, *Br J Dermatol*, **152**, 844, 2004). PBMC were isolated from whole blood by Lymphopaque centrifugation and cultured in sextuplicate in 96-well plates +/- 10µg/ml phytohaemagglutinin (PHA). PBMC proliferation was assessed at 72-80h by measurement of ^{3}H-thymidine incorporation. The supernatants from further duplicate PBMC cultures were analysed for IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-17, GM-CSF, IFNγ, MIP-1β and TNFα using a Biorad multiplex kit read on a Luminex analyser 4, 24 and 48h following PHA stimulation.

PBMC proliferation following PHA stimulation was similar in normal and PLE subjects (p=0.7) and phototherapy did not significantly alter the proliferative responses of PBMC from PLE subjects (p=0.5). With the exception of IL-8 and MIP-1β, cytokine secretion without PHA stimulation was low at all times points. PHA stimulated equivalent secretion of the majority of cytokines from PLE (pre-phototherapy) and normal subjects at 24h and 48h. However, there was significantly higher secretion of IL-4 ($p \leq 0.03$) and IL-12p70 ($p \leq 0.02$) at 24h and 48h from PBMC taken from PLE patients prior to phototherapy. A standard course of TL-01 phototherapy did not alter these raised levels of IL-4 ($p>0.2$) and IL-12 ($p>0.3$). These findings suggest that both IL-12p70 driven Th-1 responses and IL-4 dependent Th-2 responses may be exaggerated in PLE but do not indicate an overt Th-1/Th-2 imbalance in this condition.

This study was part of the European Union funded 'SUNALL' project (QLK4-CT01-00115).

P624

Erythemal sensitivity does not predict UVB-induced epidermal CD1a+ Langerhans' cell loss or caspase-3 activation in polymorphic light eruption (PLE)

Joanne Tye, Alison Blackburn, Sandra M. Winhoven, Margaret Brownrigg, Lesley E. Rhodes, Neil K. Gibbs
Dermatological Sciences, University of Manchester, Manchester, United Kingdom

Studies based on individual erythemal photosensitivity suggest that reduced photoimmunosuppression may be involved in the aetiology of PLE (e.g. Palmer and Friedmann, JID, 2004, **122**, 291; van der Pas *et al*, JID, 2004, **122**, 295). Loss of epidermal Langerhans' cells (LC) following UVB is an important event in photoimmunosuppression. In this study we investigate the relationship between erythemal sensitivity, loss of $CD1a^+$ LC and activation of the apoptotic effector, caspase-3 (c-3), in normal and PLE subjects.

9 healthy and 8 PLE subjects were irradiated on buttock skin with a series (21-200 mJ/cm^2) of broad-band (TL-12) UVB. 24h later, biopsies were taken from the 200mJ/cm^2 site and a non-irradiated site. Diastron® erythema meter readings were used to calculate $D_{0.025}$ as an objective measure of individual erythemal sensitivity. $CD1a^+$ LC were scored in epidermal sheets. Cryosections were double stained with antibodies against $CD1a^+$ and activated c-3.

UVB-induced $CD1a^+$ LC loss was similar (p=0.34) in normal (mean % loss ±se; 60.3 ± 7.1) and PLE (69.6 ± 6.2) skin. A relationship between $CD1a^+$ LC loss and individual $D_{0.025}$ was observed in normal (R^2= -0.68) but not in PLE subjects (R^2=0.03). Epidermal c-3 staining after UVB increased similarly (p=0.75) in normal (mean cells/hpf ± se; 18.2 ± 4.7; p=0.005) and PLE skin (23.0 ± 5.4; p=0.016). Caspase-3 activation correlated with individual $D_{0.025}$ in normal skin (R^2 = -0.35) but not in PLE skin (R^2 = 0.0075). Co-localisation of $CD1a^+$ was observed in 2/911 normal c-3$^+$cells and 0/787 PLE c-3$^+$cells.

In both normal and PLE skin there was little evidence that $CD1a^+$LC undergo c-3 mediated apoptosis in response to UVB. UVB-induced $CD1a^+$LC loss and c-3 activation are quantitatively normal in PLE but are not individually correlated with erythemal sensitivity. This suggests that unlike normals, erythemal sensitivity may not be a good predictor of photoimmunosuppression in PLE.

P625

Ultraviolet irradiation directly influences the structure of fibrillin microfibrils

Siobhan M. Reilly[1], Neil K. Gibbs[2], Christopher E.M. Griffiths[2], Rachel E.B. Watson[2], Michael J. Sherratt[3]
[1]Regenerative Medicine & Dermatological Sciences, University of Manchester, Manchester, United Kingdom; [2]Dermatological Sciences, University of Manchester, Manchester, United Kingdom; [3]Regenerative Medicine, University of Manchester, Manchester, United Kingdom

Age-related degenerative alterations in the biomechanical properties of dynamic tissues, such as skin, severely compromise their function. Elastic fibres, which are composed of elastin and fibrillin microfibrils, permit long-range deformability and passive recoil. Skin ageing is the sum of intrinsic time-dependent changes and extrinsic environmental effects, most notably long-term exposure to solar ultraviolet radiation (UVR). In this study we have examined whether a sub-erythemal dose of UVR can act directly on the structure of fibrillin microfibrils.

Fibrillin microfibrils were isolated by bacterial collagenase digestion/size-exclusion chromatography from photoprotected human buttock skin. Following extraction, one half of the microfibril suspension was irradiated with 50mJ/cm^2 broad-band UVR (Philips TL12, 280-400nm; MED ≈70mJ/cm^2) prior to visualisation by atomic force microscopy and mass mapping by scanning transmission electron microscopy (STEM). Morphologically abnormal microfibrils with highly diffuse interbead regions were frequently observed following UVR exposure. Compared with the non-UVR exposed microfibril population, UVR exposed microfibrils were significantly shorter with a significant mean mass loss of 350 kDa (SEM) per repeat (Student's t-test; $p<0.001$).

These data provide evidence for direct UVR-induced changes to the structure of a key extracellular matrix component. The fibrillin-1 protein is particularly rich in aromatic amino acid chromophores, including disulphide bond-forming cysteines (≈ 20%; cf. fibrillar collagens ≈ 2%), in theory making it susceptible to direct solar UVR photochemical damage and subsequent enhanced proteolysis by matrix metalloproteinases.

P626

Spectrophotometric study of antioxidant and iron binding capacity of selected flavonoid compounds

George Zonios, Aikaterini Dimou, Dimitrios Galaris
University of Ioannina, Ioannina, Greece

There is an intense interest in plant polyphenols as witnessed by the numerous papers devoted to various aspects of these compounds. The use of plants, herbs as antioxidants in processed foods is becoming of increasing importance in the food industry as an alternative to synthetic antioxidants. Phenolics are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They also have metal chelation properties. Their significance for the human diet and antimicrobial activity has been recently established. It is also known that metals are necessary for the correct function of the cells and of course human survival. Despite all these, expose to higher levels of these metals can have undesirable effects like the development of tumors. The exact molecular mechanism of carcinogenesis by metal ions is not precise but there is strong evidence that production of reactive oxygen species caused by metal ions plays a critical role in the process. Plenty of studies claim that the protective role of flavonoids is due to their ability to scavenge radicals but it could actually be that they bind metals like iron, cobalt, copper, nickel etc. All the above mentioned provided the basis for our interest in this work. All the selected flavonoids in this work had similar chemical structures differing in the number and position of –OH substitutes in the aromatic ring. In order to test their capacity to bind metals such as iron, a spectrophotometric study was carried out in the presence and absentia of iron salts in PBS (pH: 7.4) environment. All spectra obtained were analyzed and a comparison was made among them. Our findings indicated that not all of the target compounds were stable in the alkaline region while quercetin and chrysine appeared in other oxidation states causing a problem in the analysis. Some of the compounds like the 3-hydroxyflavone and 5-hydroxyflavone behaved differently in different solvents. Iron binding occurred mainly: a) between 3' –4' hydroxyl substituents and mainly at the –ortho positions in the aromatic ring, b) between the 3-hydroxyl group and 4-oxo position in the ring and not between 5-hydroxyl group and 4-oxo position. Our general remark is that flavonoid compounds with higher number of –OH groups present a higher ability in iron binding.

P627

The effects of differently coated titanium dioxide particles on UVA-induced oxidation of biological substrates

Elisabetta Venditti[1], Lucedio Greci[2], Elisabetta Damiani[1]
[1]Università Politecnica delle Marche, Istituto di Biochimica, Ancona, Italy; [2]Università Politecnica delle Marche, Dipartimento di Scienze e Tecnologie Chimiche, Ancona, Italy

Titanium dioxide (TiO_2) particles (20-50 nm in size) have long been used as physical sunscreen agents because of their ability to block solar UV light from penetrating the skin over the whole UVA/UVB range (290-400 nm) principally through reflection and scattering. Sunscreen TiO_2 is often coated with such compounds such as $Al(OH)_3$, alumina, silica and zirconia (inorganic surface treatments) which ameliorate the particles physical, chemical and heat stabilities and are of paramount importance for compatibility in formulations. Other treatments include organic surface treatments (alkoxy titanates, silanes, methyl polysiloxanes) which modify the particles' surface from hydrophilic to hydrophobic again benefiting the particles' physical and chemical stability and promoting wetting and steric stabilization in a carrier. Doping transition metal ions into TiO_2 is another recent treatment used to modify its photocatalytic activity. Besides reflecting and scattering UV light, TiO_2 also absorbs it efficiently, catalysing the formation of superoxide and hydroxyl radicals which can initiate oxidations that could account for the photo-toxicity of illuminated TiO_2. Since one of the most critical factors influencing the photo-activity of TiO_2 appears to be the nature of the surface, it is plausible to expect that different surface treatments will affect its photocatalytic activity as has already been observed with some. The present study was undertaken to compare the photo-oxidative effects, or lack thereof, of seven differently coated or doped TiO_2 specimens from various commercial sources, in contact with different biological substrates upon UV-A illumination. Three different concentrations for each specimen were tested on UV-A induced oxidation of liposomes (lipid substrate), bovine serum albumin (protein substrate) and deoxyribose (sugar substrate, component of DNA backbone). The results obtained differed according to the substrate used and, as expected, according to the type of treatment. In general, increased oxidation was observed with deoxyribose, less so with BSA while no additional UV-A induced oxidation was detected with liposomes. The findings highlight the fact that some TiO_2 surface treatments are more effective than others at promoting photo-oxidative events when in contact with biological substrates, especially with more hydrophilic ones such as water-soluble proteins and sugars and this may have implications concerning their safe use in sunscreens and cosmetics in general.

P628

Effects of the flavonoid luteolin on the human sunburn response

Lien Verschooten[1], Katrien Smaers[1], Lieve Declercq[2], Daniel Maes[2], Marjan Garmyn[1]
[1]Laboratory of Dermatology, University of Leuven, Leuven, Belgium; [2]Estee Lauder Compagnies, Oevel, Belgium

UV-exposure of the skin may lead to the formation of sunburn (or apoptotic) cells and the induction of erythema, known as sunburn. Both reactive oxygen species (ROS), vasoactive and proinflammatory mediators are involved in the sunburn response. The flavonoid Luteolin is known as an excellent antioxidant, and exhibits in some cell types and tissues also anti-inflammatory characteristics. We have shown previously that Luteolin increases the resistance of normal human epidermal keratinocytes (NHEK) to UVB. In this study, we investigate the capacity of Luteolin in modulating important mediators of the sunburn response: ROS, the vasoactive mediator prostaglandin (PG)-E2 and the proinflammatory cytokine interleukine (IL)-1alpha. FACS detection of ROS using the DCF-DA dye revealed an inhibition of UVB-induced ROS by Luteolin. Moreover, also basal ROS-level in sham-irradiated, Luteolin-treated NHEK was diminished. As ROS is known to arouse the mitochondrial apoptotic pathway, depolarization of mitochondrial membrane potential (MMP) was checked with flow cytometry using DIOC6. Luteolin (5 & 10 microM) was seen to inhibit loss of MMP after UVB. The effect of Luteolin on the release of PGE2 and IL1-alpha in the supernatans of UVB-irradiated NHEK, was investigated with ELISA. Both UVB-induced release of IL-1alpha and PGE2 was almost completely inhibited after Luteolin (1 and 5 microM) treatment. These results indicate that Luteolin is able to modulate the acute UVB-response in NHEK via its antioxidant and anti-inflammatory effects.

P629

Which hazard weighting function should be used for assessment of eye exposure from dental radiation sources?

Ellen M Bruzell[1], Bjørn Johnsen[2], Tommy Nakken Aalerud[2], Terje Christensen[2]
[1]Nordic Institute of Dental Materials (NIOM), Haslum, Norway; [2]Norwegian Radiation Protection Authority (NRPA), Østerås, Norway

Lamps used to polymerise white tooth filling materials and lamps used in tooth bleaching processes in the dental clinic emit mostly blue light. Intensities of dental lamps have increased by a factor 10 over the last decade and now typically have values of $1 W/cm^2$, but

certain lamps emit up to three times this intensity. Depending on the source, some lamps emit UV radiation from a small part of the spectrum in addition to visible light. There is a common misunderstanding that emission from dental curing lamps requires mainly UV eye protection. Consequently, there are sold dental curing lamp eye protectors unsuitable for its purpose. Products intended for personal eye protection against noncoherent optical radiation shall be marked according to relevant standards. We assessed the lamps' eye hazard potential using ultraviolet and blue-light hazard weighting functions (ICNIRP and ACGIH). Emission spectra of 26 dental curing and bleaching lamps were characterised and transmittance spectra of eighteen eye protective filters were measured. The maximum permissible exposure duration (t_{max}) (ACGIH) with and without filter was estimated based on the light flux, spectral reflectance, area of the tooth and eye and the distance between the tooth and eye of the operator. The t_{max} values obtained using blue-light hazard weighting function were lower than those obtained using ultraviolet hazard weighting function for all lamp sources. The ultraviolet eye hazard from the investigated dental sources was insignificant for all but one lamp. Nine of the 18 tested filters had adequate filtering capacity according to today's lamp technology and exposure limit values for blue light. These filters transmitted less than 0.1% of the radiation at any wavelength between 400 nm and 525 nm. One-third of the filters lacked marking. A majority of the marked filters were insufficiently marked or were marked according to an irrelevant standard. In addition, some filters successfully tested and marked according to relevant standards did not protect throughout an 8-hour work day. We suggest that amendments are made to relevant standards to account for new lamp technology and the significance of the blue light hazard function.

P630

Misuse of light in tooth bleaching

Ellen M Bruzell[1], *Jon E Dahl*[1,2]

[1]*Nordic Institute of Dental Materials (NIOM), Haslum, Norway;* [2]*Faculty of Odontology, University of Oslo, Oslo, Norway*

Tooth bleaching has gained wide popularity, and procedures are offered by dental clinics, beauty parlours, and hairdressers. Bleaching products are also available for at-home application. Most products are based on carbamide- or hydrogen peroxide as the bleaching ingredient. In recent years, light is sometimes added to the bleaching procedure. Most of the commercial bleaching lamps emit in the 390-550 nm wavelength range with short and long wavelength extreme values of 350 and 740 nm, respectively. Irradiance values vary between 10-2800 mW/cm^2. The study was undertaken to determine whether light-assisted bleaching gave any additional bleaching effect to the bleaching agents used alone. Seven bleaching systems were investigated on extracted human teeth. Each tooth crown was split in two parts; one part was bleached and the other served as control. A number of 10-20 teeth were exposed to each bleaching product with or without additional light exposure. Tooth lightening effect was determined immediately after procedure and again after 1 week. Bleaching with light did not result in statistically significant lightness after 1 week compared with teeth bleached without light. Immediate inspection showed significant increase in lightness for one of the seven systems. Our finding of no additional light-effect in tooth bleaching procedures is supported by a majority of other clinical and laboratory studies. We have estimated that the maximum permissible exposure time (based on ICNIRP) of unprotected oral tissues and skin by UV radiation from a particular bleaching lamp is 6 min, while procedures lasts up to 1 hour. Furthermore, estimated maximum permissible exposure times (based on ICNIRP) of unprotected eyes by blue light is 6-20 min depending on the lamp. Since the use of bleaching light does not result in improved tooth lightening and the exposure dose limits are exceeded during treatment we will advise against the use of light-assisted tooth bleaching.

P631

Spectroscopic and electrochemical properties of 2-aminophenothiazine in acetonitrile

Rolando Oyola[1], *Carmelo García*[1], *Luis Piñero*[1], *Ileana Nieves*[1], *Nadya Cruz*[1], *Rafael Arce*[2]

[1]*University of Puerto Rico-Humacao, Humacao, Puerto Rico;* [2]*University of Puerto Rico-Río Piedras, San Juan, Puerto Rico*

Phenothiazines (PH) and related derivatives are used in many applications including medicinal chemistry, polymer chemistry, sensors, and material sciences. The participation of the excited triplet state and/or the redox properties of PH derivatives directly affect the effectiveness of these areas. Although the photophysical and photochemical properties of the 3-substituted derivatives have been studied extensively, there is very few knowledge regarding the 2-position derivatives. In this work, we present the photophysical and photochemical properties of 2-aminophenothiazine (APH) in acetonitrile solution. Aminophenothiazine showed a very weak fluorescene emission (φ_f =0.0025) centered at 450 nm with a lifetime of 650 ps. Characterization of the 355 nm nanosecond laser flash photolysis transient species reveals the presence of the triplet-triplet transient intermediate with high formation yield. In aerated acetonitrile solution, APH sensitized the formation of excited singlet oxygen. Therefore, APH is a good candidate as a photosensitizer.

This work is sponsored by NIH-S06GM08216.

P632

Photoreactivity of indirubin derivatives

David Olivier, *Samuel Douillard, Thierry Patrice*

Cancer Photobiology, HGRL, Nantes, France

Introduction: Indirubin (isomer of indigo) analogs have been synthesized to optimize their promising kinase inhibitor scaffolds. As they are pigmented it appeared interesting to test their photoreactivity under light exposure at an appropriate wavelength to anticipate adverse reactions for those of compounds that would be clinically developed. A therapeutic strategy could also combine the tumor growth inhibition observed with kinases inhibitors with the massive blood flow arrest observed after photodynamic reaction and used clinically.

Materials & Methods: 29 very similar indirubinoids were first screened using an original high performance chemical ROS test. Influence of serum on absorption and fluorescence spectra were recorded. Toxicity and phototoxicity were then assessed on F98 cells (a chemo- and radio- resistant murine cell line).

Results & Conclusions: Absorptions were comprised between 450 nm and 620 nm, and modified by serum for 4 compounds. Fluorescence (comprised between 600 and 680 nm) increased in 13, decreased in 6, remained unaltered in 10, after FCS (10%) addition. ROS production although less than with classical sensitizers was found to be significant in 11 compounds and proportional to fluorescence detected. Phototoxicity was good compared to toxicity for 7 compounds. None of the observed pattern could be related to any of the structural changes of the tested chemicals. No structural criterion predicting photoreactivity was evidenced suggesting that techniques of massive screening have to be developed and validated. The approach consisting in detecting new sensitizers among chemicals of natural origin, combined to new pharmaceutical properties could be a promising one for PDT.

P633

Photoredox processes in synthetic nucleosides: intramolecular photoreactions of ketoprofen-purine dyads

Cecilia Paris[1], *Noureddine Belmadoui*[2], *María José Climent*[3], *Susana Encinas*[3], *Miguel Ángel Miranda*[1]
[1]*Instituto de Tecnología Química UPV-CSIC, Universidad Politécnica de Valencia, Valencia, Spain;* [2]*CEA Grenoble, DRFMC/SCIB/LAN, Grenoble, France;* [3]*Departamento de Química, Universidad Politécnica de Valencia, Valencia, Spain*

Although design and development of drugs are aimed at minimizing the occurrence of side-effects, in the last years an increasing number of photosensitivity disorders have been reported. These are abnormal skin reactions appearing upon exposure to sunlight.

Ketoprofen (KP) and related non-steroidal anti-inflammatory agents exhibit photosensitizing properties. In particular, KP causes oxidative DNA damage and also energy transfer-mediated thymidine dimer formation. Most of the studies have dealt with pyrimidine nucleosides; by contrast purine bases, their nucleosides, and nucleotides have received much less attention. Thus, the photosensitization mechanisms still remain uncertain for purine derivatives.

In this work, time resolved experiments and steady-state irradiation have been performed on synthetic dyads with a KP unit tethered to the C3' and C5' positions of 2'-deoxyguanosine (dG) and 2'-deoxyadenosine (dA).

Selective excitation of the benzophenone-like chromophore at λ= 355 nm or λ= 308 nm allowed the detection of the triplet excited state and the ketyl radical of KP at λ= 530 nm and λ= 560 nm, respectively. The latter arises from protonation of a benzophenone-like radical anion, formed by a fast electron transfer process from the base to the excited KP chromophore. Deprotonation of the base radical cation leads partially to the C'1 sugar radical in competition with back electron transfer.

Photoproduct characterization revealed a variety of oxidation products including 2'-ribonolactones and deamination products.

P634

Valence isomerization of hinokitiol under UV-irradiation

Koh-ichi Seki[1], *Michiko Maekawa*[1], *Shigeru Kasahara*[1], *Shingo Nakamura*[2], *Toshihiro Okabe*[3], *Tadamiti Yamamoto*[4], *Yoshihiro Inamori*[5], *Naomi Shishido*[6], *Masao Nakamura*[6]
[1]*Central Institute of Isotope Science, Hokkaido University, Sapporo, Hokkaido, Japan;* [2]*Aomorichuogakuin University, Aomori, Aomori, Japan;* [3]*Aomori Industrial Research Center, Hirosaki, Aomori, Japan;* [4]*Hiroka R and D, Hirosaki, Aomori, Japan;* [5]*Osaka University of Pharmaceutical Sciences, Takatuki, Osaka, Japan;* [6]*Asahikawa Medical College, Asahikawa, Hokkaido, Japan*

Hinokitiol (4-isopropyltropolone)(HT), a constituent of Japanese cypress and western red cedar, has been confirmed to have bactericidal, antifungal, and anti-oxidative activities. Hinokitiol has an absorption peaks in UV region at neutral pH and is photosensitive to UV light irradiation. Formation of reactive oxygen species and HT derived products during the irradiation has been reported.[1] These studies have been made only on the basis of spectroscopic studies and no photochemical behavior of HT has been elucidated.

In order obtain more insight into the photoreaction of HT and the biological activities of products. Upon UV-irradiation of a solution of TH in methanol with a 500W high pressure Hg lamp, HT was readily converted into a mixture of valence isomers. HPLC of the mixture afforded a and the isomer b as the major products, whose structures were determined as 1-hydroxy-6-(iso-propyl)bicyclo[3.2.0]hept-3,6-dien-2-one (a) and its *trans*-isomer (b) on the basis of spectroscopic analyses.

Similar photolysis of HT under aerated conditions showed essentially analogous products pattern on the ^{1}H-NMR spectrum, suggesting that oxygen may not participate in the photo-degradation of HT.

The sodium salts of HT was photoresistant, yet afforded similar results under prolonged UV-irradiation.

The present study may suggest that oxygen may not importantly be involved in the photo-degradation of HT.

[1]H. Shibata, T. Nagamine, Y. Wang, T. Ishikawa, and Y. Sawa, Biosci. Biotechnol, Biochem., **67**,1996-1998 (2003).

P635

Diastereoselective photocycloaddition of nucleoside aanalogues with naphthalene

Koh-ichi Seki[1], *Masayuki Takahashi*[2], *Ken-ichi Nishijima*[2], *Kazue Ohkura*[2]
[1]*Central Institute of Isotope Science, Graduate School of Medicine, Radio Biology, Hokkaido University, Sapporo, Japan;* [2]*Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Japan*

In our continuing studies, we have revealed that UV-irradiation of 5-fluoro-1,3-dimethyluracil with naphthalene and its derivatives in an aprotic medium afforded ethenoquinazoline (barrelene) derivatives in high yield with good regio- and stereo-selectivity. Enantioselective or diastereoselective photoreactions, such as [2+2]- and [4+2]-cycloadditions are established tools in organic synthesis. Hence, our attention was focused on the possible diastereodifferentiating photocoupling of 5-FU derivatives with naphthalenes by introducing an asymmetric center at N1 of the uracil ring. In this paper we report that UV-irradiation of O-acetyl-5-fluorouridine and its derivatives having an asymmetric carbon attached to N1 undergoes diastereoselective photo-Diels-Alder reaction with naphthalenes, to give ethenobenzoquinazolines in good yields.

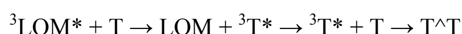
Irradiation of a solution of a 5-fluorouridine derivative and naphthalene in benzene in a de-gassed Pyrex test tube with a high-pressure mercury lamp (>300 nm) afforded a separable mixture of diastereomeric 4a-fluoro-5,10-ethenobenzo[*f*]-quinazolines in high yield. In the case of tegafur, HPLC of the mixture furnished the 2'*R*- and the 2'*S*-diastereomer in 72.5 % and 17 % yield, respectively. The configuration of the resulting adducts is unambiguously derived from X-ray crystal structural analysis. From a viewpoint of molecular chirality controlling photoreaction, it may be noteworthy that the present work provided the first clear example of diastereoselective photo-Diels Alder reaction in the nucleic acid chemistry.

P636

The triplet properties of the photocarcinogenic fluoroquinolone antibiotic, lomefloxacin, are not consistent with an energy transfer mechanism for photosensitised thymine dimer formation

Kristy Clarke[1], *George Truscott*[1], *David J. McGarvey*[1], *Neil K. Gibbs*[2]
[1]*School of Physical & Geographical Sciences, Keele University, Keele, UK;* [2]*Dermatological Sciences, University of Manchester, Manchester, UK*

In common with several fluoroquinolone antibiotics, lomefloxacin (LOM) photosensitizes human skin, is photogenotoxic and is highly photocarcinogenic in mouse skin. We have demonstrated that LOM photosensitizes mutagenic thymine dimer (T^T) formation *in vitro* (Traynor & Gibbs, *Photochem Photobiol*, 70, 957-9, 1999) and it has been recently reported that mice lacking DNA excision repair of T^T (XP-A) are exquisitely sensitive to LOM photocarcinogenesis (Itoh *et al*, *J Invest Dermatol*, 125, 554-9, 2005). The classic mechanism for photosensitized T^T formation is via energy transfer from the triplet excited state of the photosensitiser (^{3}LOM*) to a thymine molecule (T) which forms ^{3}T* which can then dimerise with an adjacent T:



$^3LOM^* + T \rightarrow LOM + {}^3T^* \rightarrow {}^3T^* + T \rightarrow T\hat{}T$

An absolute requirement for this reaction is that the triplet state energy (E_T) of $^3LOM^*$ exceeds that of $^3T^*$ (308 kJ.mol^{-1}). We used the time-resolved technique of laser flash photolysis (LFP) to study the properties of $^3LOM^*$. LOM triplets could not be directly observed using LFP, which concurs with previous reports of short $^3LOM^*$ lifetimes. We therefore investigated whether LOM could receive transferred energy, and hence quench, LFP-generated triplets of biphenyl ($^3BIP^*$, E_T=274 kJ.mol^{-1}) or naphthalene ($^3NAP^*$, E_T=253 kJ.mol^{-1}). LOM was not able to quench $^3NAP^*$ suggesting that $^3LOM^*$ has an $E_T > 253$ kJ.mol^{-1}. Conversely, LOM was able to quench $^3BIP^*$ suggesting that $^3LOM^*$ has an $E_T < 274$ kJ.mol^{-1}.

These results indicate that the $^3LOM^*$ E_T is in the range 253-274 kJ.mol^{-1} and well below the E_T for $^3T^*$ (308 kJ.mol^{-1}). This finding makes energy transfer from $^3LOM^*$ to T an energetically unfeasible route for T^T formation. We are currently exploring other mechanisms by which LOM photosensitizes T^T formation including possible triplet energy transfer from LOM photoproducts.

P637

Photosensitization of cholesterol by diaryl ketones in model dyads

Inmaculada Andreu, Isabel M. Morera, Francisco Boscá, Miguel A. Miranda

Departamento de Química, Universidad Politécnica de Valencia, Valencia, Spain

Cholesterol (Ch) is one of the most important structural components of cell membranes and is a major target for oxidative degradation, a process which can result in potentially pathologic consequences. Two mechanisms have been considered for Ch oxidation: Type I (*via* free radicals) and Type II (mediated by $^1O_2$). Both mechanisms can be promoted, in principle, by UVA-irradiation in combination with photosensitizing agents.

Ketoprofen (KP) and tiaprofenic acid (TPA) are benzophenone(Bz)-derived non-steroidal anti-inflammatory drugs (NSAID) with proven photosensitizing potential. The former displays a lowest-lying triplet excited state n,π* and, accordingly, is a typical Type I photosensitizer. In contrast, TPA (which contains the 2-benzoylthiophene chromophore) has a π,π* lowest triplet excited-state and thereby the contribution of type II oxidation mechanism is enhanced.

In the present work, several dyads have been synthesized from β- and α-Ch and KP or TPA as models to study the potential mechanism of Bz-photosensitized Ch oxidation. These dyads have been submitted to time-resolved and steady-state photolysis studies. Upon irradiation, (*S*)-KP-α-Ch and (*R*)-KP-α-Ch were photolyzed very quickly, affording well-defined photoproducts *via* intramolecular hydrogen abstraction from C-7 and subsequent C-C coupling of the generated biradicals, whereas (*S*)-KP-β-Ch, (*R*)-TPA-α-Ch, (*S*)-TPA-α-Ch, and TPA-β-Ch remained unchanged after prolonged irradiation. Hence the photoreactivity of the dyads was markedly dependent on the electronic nature of the excited states (n,π* vs. π,π*).

The transient absorption spectra (λexc= 355 nm) of (*S*)-KP-α-Ch and (*R*)-KP-α-Ch were very similar; they did not correspond to their triplet excited states but to the corresponding biradicals. Interestingly, the lifetimes of the diastereomeric biradicals were significantly different (280 ns for (*S*)-KP-α-Ch *vs* 220 ns for (*R*)-KP-α-Ch). By contrast, the transient absorption spectra and lifetimes obtained for all the TPA-derived dyads were very similar to that of the tiaprofenic acid triplet excited state.

P638

Photophysical and photochemical characteristics of 3-nitrofluoranthene in solution

Salvador D Gavalda, Rafael Arce

University of Puerto Rico - Rio Piedras, San Juan, PR, United States

Understanding the photophysical and photochemical properties of nitrated polycyclic aromatic hydrocarbons (NPAH) is important because these compounds are found in air particulate matter and combustion emissions. Some NPAHs have been observed to be more mutagenic than the PAH from which they are derived, and some have been demonstrated to have a carcinogenic potential in animals. Also, the photostability of NPAHs seems to depend on the physical and chemical nature of the compound on which NPAHs are adsorbed.

By using solvent media can be used to simulate the organic component of aerosols, further insight can be obtained about the photodecompostition process of 3-nitrofluoranthene. This also allows comparing the photophysical and photochemical properties of the nitrated and non-nitrated PAH. Once studies of 3-nitrofluoranthene photochemistry are completed and photoproducts determined, photoproduct toxicity can be compared with that of its parent molecule. The absorption and fluorescence spectra and fluorescence quantum yields were determined for 3-nitrofluoranthene dissolved in polar, non-polar, and proton-donating solvents. The Stokes shift of 3-nitrofluoranthene was greatly affected by solvent as calculated shifts were 3nm when dissolved in hexane, 79 nm in methanol and 213 nm in acetonitrile. Meanwhile, the observed Stoke Shift of the non-nitrated fluoranthene was not affected by solvent. Fluorescence quantum yield of 3-nitrofluoranthene was calculated relative to reference value of the quantum yield of fluoranthene, which has an estimated fluorescence yield coefficient of 0.35 in nonpolar solvent. When compared to 3-nitrofluoranthene, the differences were almost of two complete orders of magnitude. Fluorescence yield coefficients for 3-nitrofluoranthene were estimated to be 0.0054 in methanol solvent and 0.0057 in hexane.

P639

The poor teacher's spectrophotometer for *in vitro* and *in vivo* absorption and fluorescence spectra

Lars Olof Bjorn

Lund University/Cell and Organism Biology, Lund, Sweden

A spectrophotometer for student experiments and demonstrations can be constructed very easily and at relatively small cost if you have already got a simple digital camera, a computer, a DVD that can be used as a grating and a light source. The wavelength range of the spectrophotometer is 400 to 700 nm, and the wavelength resolution 4 – 5 nm. The instrument can be used not only for recording absorption spectra of clear solutions, but also for spectra of scattering samples such as plant leaves. In addition it can be used for recording fluorescence spectra. Absorption spectra of substances separated by paper chromatography or paper electrophoresis can be recorded directly from the paper without elution. The spectrophotometer can also be adapted for recording reflectance spectra.

Software freely available from the Internet is used for data processing and plotting of spectra, and results can be displayed in the classroom by use of a computer projector. Data can also be transferred to Excel or Powerpoint. Because the equipment is so cheap, it is possible to have many sets in the class.

The possibilities of recording, in a simple way, the scattering and fluorescence changes associated with high light adaptation in a plant leaf are being explored. The best option for this seems to be to use a green laser pointer as light source.

P640

James Prescott Joule (1818 - 1889): a Manchester son and the father of the international unit of energy

Sandra M Winhoven, Neil K. Gibbs

Dermatological Sciences, University of Manchester, Manchester, United Kingdom

All those involved in photobiology will know of the the 'Joule' but perhaps are not familiar with the man who gave his name to this international unit of energy. James Prescott Joule was born on Christmas Eve 1818 into a wealthy Manchester (UK) brewing family. He was tutored at home where he indulged his fascination for electricity by using a voltaic cell to give electric shocks to the family servants! At the age of 16 he was sent to the Manchester Literary and Philosophy Society (*Lit & Phil*) to be taught chemistry, physics, mathematics by the eminent Manchester scientist, John Dalton (1766-1844; developer of the atomic theory of matter).

Joule managed the family brewery from 1837 to 1856 which enabled him to experiment on the relationships between heat and electricity in a laboratory built in the cellar of his father's home. In 1840, Joule published a paper in the *Proceedings of the Royal Society* describing the first of the eponymous laws which predicts the heat generated by a conductor from its resistance and the current applied. In 1843 he showed that heat was a form of energy and determined the physical constant now used as the S.I. unit for energy, the Joule (J). He demonstrated the mechanical equivalent of heat by measuring change in temperature caused by the friction of a paddlewheel attached to a falling weight. Up to then most scientists believed in the caloric theory that heat could neither be created not destroyed. Joule's theory was so controversial that initially, no scientific journal would publish it. However, William Thompson (later Lord Kelvin) supported Joule and collaborated with him to examine heat changes when gases expand and contract which led to the development of the refrigerator. Joule also invented 'arc' welding, and the displacement pump. Despite carrying out admirable research to improve the quality of beer, in 1875 Joule's funds ran out. With the support of members of the *Lit & Phil*, Joule was granted a Civil List pension of £200 p.a. for services to science. Joule died on 11 October 1889 and his gravestone is inscribed with the number 772.55, his 1878 determination of the weight in pounds that could be lifted one foot by the same amount of energy required to heat one pound of water by one degree. Joule never took an academic appointment but was made FRS (1850) and received honorary doctorates from Dublin, Oxford and Edinburgh. He is commemorated on a memorial in Westminster Abbey, London and a statue of James Prescott Joule stands opposite that of his former tutor, John Dalton, in the entrance to Manchester Town Hall.

P641

Nanocluster photocatalytic degradation of some organic pollutants

Souad Ahmed Elfeky, Alsayed Abdelmajid Alsherbini

Cairo University/National institute of laser enhanced sciences, Giza, Egypt

Contamination of water by organic dyes presents a serious environmental risk because of their toxicity and resistance to degradation. The goal of this research is to enhance the photodegradation of these organic pollutants using low cost, non toxic, and strong oxidizing power nanoparticles. The effect of semiconductor catalysts as TiO_2 was studied.

Keywords: Nanocluster, photodegradation & organic dyes.

P642

Photoinactivation of bacteria in wastewater by porphyrins. Bacterial β-galactosidase activity and leucine-uptake as methods to monitor the process

C.M B. Carvalho[1], A.T.P.C. Gomes[1], S.C.D. Fernandes[2,3], A.C.B. Prata[3], M.A. Almeida[3], M.A. Cunha[3], J.P.C. Tomé[1], M.A.F. Faustino[1], M.G.P.M.S. Neves[1], A.C. Tomé[1], J.A.S. Cavaleiro[1], Z. Lin[1,4], J.P. Rainho[4], J. Rocha[4]

[1]University of Aveiro, Department of Chemistry, 3810-193 Aveiro, Portugal; [2]University of Aveiro, Department of Biology, 3810-193 Aveiro, Portugal; [3]University of Aveiro, CESAM, 3810-193 Aveiro, Portugal; [4]University of Aveiro, CICECO, 3810-193 Aveiro, Portugal

The increasing interest in photodynamic antimicrobial chemotherapy as a promising technology for wastewater treatment demands more efficient photosensitizers and faster methods for the monitorization of the photoinactivation process. In this communication we will describe the results obtained when several cationic porphyrins were used as photosensitizers for the photoinactivation of sewage bacteria. Two of these porphyrins revealed to be highly active, even against Gram-negative bacteria, inactivating ca. 94-99.8% of these at 5 μM and after 270 minutes of irradiation with white light (9 $mW.cm^{-2}$). In addition, two expeditious methods for the evaluation of the bacteria photoinactivation were developed[1]. These methods are based on β-galactosidase activity and on leucine incorporation and give an excellent relation with faecal coliform counts. The evaluation of these two descriptors of bacterial activity, directly related with bacterial abundance, is an easy and fast way to monitor the micro-organisms during the photoinactivation process.

[1]Carvalho CMB, Gomes ATPC, Fernandes SCD, Prata ACB, Almeida MA, Cunha MA, Tomé JPC, Faustino MAF, Neves MGPMS, Tomé AC, Cavaleiro JAS, Lin Z, Rainho JP, Rocha J Journal of Photochemistry and Photobiology B: Biology, in press.

Thanks are due to the University of Aveiro, Fundação para a Ciência e a Tecnologia (FCT) and POCI 2010 (FEDER) for funding the project POCI/CTM/58183/2004. CMB Carvalho, ATPC Gomes and JPC Tomé are also grateful to FCT for their grants.

P643

Photochemical degradation of phenylurea herbicide chlorotoluron

Sarka Klementova, Martina Zemanova

Fac. Biol. Sci., Univ. South Bohemia, Ceske Budejovice, Czech Rep.

Phenylurea herbicide chlorotoluron is a herbicide widely used for weed control in wheat production. It belongs to the group of herbicides which are chemically stable and relatively resistent to microbial degradation. In our study, we focused on photochemical degradation of chlorotoluron in homogeneneous solution. UV light (250 – 350 nm, maximum emission at 300 nm) was used for irradiation. Influence of solvent (water, water – methanol mixture, methanol), metal ions (ferric ions) and humic substances on the degradation kinetics was investigated. Production of reactive oxygen species (hydroxyl radicals) during irradiation and product formation, namely the extent of total mineralization, was estimated. Reaction rate constants and quantum yields of the reactions were calculated.

P644

Bioluminescent control of sterility

Martina Bancirova, Hana Fabianova

Palacký University, Department of Physical Chemistry, Olomouc, Czech Republic

The monitoring of sterility is important for different types of our surroundings. It is very significant to obtain the real-time results of total bacterial contamination level.

The classical microbiological methods need only simple equipment but the realization takes too long time, usually two or three days. During recent years, luminescent analysis has become widely used as a replacement or alternative method for many assays carried out using such conventional techniques. The advantages that luminescence has over methods based on light absorption are higher sensitivity, wider measurement range and smaller specimen samples.

Bioluminescence is the term applied to chemiluminescent reactions that occur naturally in light-emitting organisms such as fireflies, jellyfish, fungi, marine bacteria, etc. The oxidoreduction step of such reactions is catalyzed by enzyme called luciferase. Bioluminescence is the most efficient light-producing system because the enzymes specifically direct the reaction through a light-emitting pathway. The measurement of ATP is based on the luminescent reaction-firefly bioluminescence. Firefly luciferase enzyme catalyzes the activation of the substrate D-luciferin by ATP and its subsequent oxidation to excited oxyluciferin. The presence of Mg^{2+} ions is also required to initiate luciferase activity. The transition of excited oxyluciferin to its ground state results in light emission. The reaction conditions are designed so that the emitted light is directly proportional to ATP concentration, in principle, any substance linked to the production or consumption of ATP can be quantified. All living cells contain ATP. When cells die of natural causes, ATP is normally degraded. Most bacterial cells contain approx. 2.10^{-18} mol ATP per cell. The results of determinations of total bacterial contamination were assigned in CFU/100 cm^2. CFU means colony forming units.

The aim was the bioluminescent assay of sterility of various types of surfaces in a hospital environment (e.g. clean bed-clothes, hands of personnel, saucer for patient items, table for not sterile items, manipulation table).

P645

Kinetic properties of bacterial luciferases in water-organic solvents

Irina E. Sukovataya, *Elisaveta V. Kaykova*

Department of Biophysics, Krasnoyarsk State University, Krasnoyarsk, Russia

Luciferases of luminous bacteria - flavin-dependent monooxigenases catalyze the oxidation reaction of the long-chain aliphatic aldehyde and reduced flavinmononucleotide ($FMNH_2$) involving molecular oxygen to a respective fatty acid emitting light quanta in the visible spectrum. Initiated by photoreduced $FMNH_2$, the bioluminescent reaction is a short flash of light with pronounced maximum and fast decay of bioluminescence; the enzyme makes, at this, one cycle. The rate constant of bioluminescent decay (k), maximum emission intensity (I_{max}) and quantum yield (Q) in the presence of organic solvents were measured. This work presents results on examination of the dielectric permittivity effect on the kinetic parameters of luciferases from two types of luminous bacteria *Photobacterium leiognathi* and *Vibrio harveyi*. There were three solvent types changing the dielectric permittivity of the medium (ε) to a different degree: 1) ε-decreasing solvents (ethanol, methanol, acetone and ethylene glycol), 2) ε-increasing solvent (formamide), 3) a solvent slightly (compared to the buffer solution) changing ε (dimethyl sulfoxide - DMSO). Used solvents are water-soluble and relatively hydrophilic with hydrophobicity values < 2. With addition of solvents when ε ranges from 74 to 82 increases of the I_{max} and the Q depend on the balance between the capacity of the solvent to form hydrogen bonds and moderate hydrophobic contacts. Addition of formamide attenuates electrostatic interactions and increases the decay rate of excited emitter. High electron-donor capacity of DMSO and acetone helps stabilize the excited intermediate of the reaction. In many cases, the stabilization of the excited intermediate observed for luciferase from *V. harveyi* in the presence of organic solvent was greater in the case of enzyme from *P. leiognathi*. It is shown that stabilization of the excited intermediate varies with the solvent but did correlate with a type of specific binding of aldehydes with luciferases. The luciferase from *V. harveyi* is more stable in the presence of organic solvents and may prove valuable for catalysis in such environments with the higher light emission. Methanol, ethanol and formamide are the solvents of choice to enhance enzymatic activity. Such studies are designed to answer questions concerning the quantitative aspects of the biotransformation process and the applied aspects of bioluminescence.

This work was made possible in part by Award No. 07-04-01340a of the Russian Foundation for Basic Research.

P646

Differences in processing of blue light signals between animal and plant cryptochromes

Tilman Kottke[1,2], *Alex Berndt*[3], *Helena Breitkreuz*[3], *Joachim Heberle*[1], *Margaret Ahmad*[4], *Eva Wolf*[3]

[1]*Biophysical Chemistry, Bielefeld University, Bielefeld, Germany;* [2]*INB-2: Molecular Biophysics, Research Center Juelich, Juelich, Germany;* [3]*Max-Planck-Institute of Molecular Physiology, Dortmund, Germany;* [4]*Universite Paris VI, Paris, France*

Cryptochromes are blue-light photoreceptors that regulate several responses in *Arabidopsis* such as de-etiolation and flowering time[1]. In *Drosophila*, a single animal cryptochrome entrains the circadian rhythm of the fly[2]. All cryptochromes contain a photolyase homology region (PHR) of about 500 amino acids and a C-terminal extension of varying length. In the PHR domain, flavin adenine dinucleotide is noncovalently bound as chromophore. The pathway from light absorption to signal transduction in cryptochromes is poorly understood.

In *Arabidopsis* cryptochrome 1, illumination induces electron and proton transfer to the oxidized flavin with formation of a neutral flavoprotein radical, the putative signalling state. The spectral features of this radical clearly distinguish it from that of structurally highly homologous DNA photolyases[3]. In contrast, absorption of blue light by *Drosophila* cryptochrome leads to formation of an anionic flavoprotein radical, characterized by a strongly different absorption spectrum[4]. The conversion proceeds independently of the presence of external electron donors. In the dark and under aerobic conditions, the reaction is fully reversible in the time scale of minutes. We propose that the anionic radical state mediates resetting of the circadian clock in *Drosophila*. These findings demonstrate that animal and plant cryptochromes do not share a common mechanism of light activation.

[1]Lin C. (2002) *Plant Cell 14*, S207-S225. [2]Stanewsky R., Kaneko M., Emery P., Beretta B., Wager-Smith K., Kay S.A., Rosbash M., Hall, J.C. (1998) *Cell 95*, 681-692. [3]Kottke T., Batschauer A., Ahmad M., Heberle J. (2006) *Biochemistry 45*, 2472-2479. [4]Berndt A., Kottke T., Breitkreuz H., Dvorsky R., Hennig S., Alexander M., Wolf E. (2007) *J. Biol. Chem. 282*, 13011-13021.

P647

A blue light inducible two component signal transduction system in the plant pathogen *Pseudomonas syringae* pv. tomato

Zhen Cao[1], *Valentina Buttani*[2], *Aba Losi*[2], *Wolfgang Gaertner*[1]

[1]*Max Planck Institute for Bioinorganic Chemistry, Muelheim an der Ruhr, Germany;* [2]*Dept. of Physics, University of Parma, Italy, Parma, Italy*

LOV- (light, oxygen, voltage) domain is one of the most spread motifs for flavin-binding, blue light-sensitive photoreceptors in bacteria (found in 15 – 20 % of all sequenced genomes). Upon UVA/blue-light irradiation, it binds flavin mononucleotide (FMN) as chromophore and undergoes a photo-cycle involving an intermediate with the chromophore covalently attached to the protein. LOV domain is linked to a variety of signal-output domains, some of which are already well known from other signaling pathways, e.g. the histidine kinase (HK) and response

regulator (RR) motif, which is a typical two-component signaling pathway in bacteria.

The open reading frame *PSPTO2896* from the plant pathogen *Pseudomonas syringae* pv *tomato* encodes a protein of 534 amino acids, which contains an N-terminal LOV domain and a C-terminal HK-RR motif, showing all salient features of a blue light-driven two component system. The HK and RR can be phosphorylated at a conserved His and Asp residues, respectively. Phosphor-transfer from the HK to the RR results in activation of the RR and generation of the output response of the signaling pathway.

The full length protein (PST-LOV) and, separately, the RR and the LOV+HK part (PST-LOVΔRR) were heterologously expressed and functionally characterized. The two LOV proteins showed typical LOV-like spectra and photochemical reactions, with the blue light-driven, reversible formation of a covalent flavin-cysteine bond. The fluorescence of the single tryptophan in LOV domain changes in the lit state for PST-LOV, but not in PST-LOVΔRR, indicating a direct interaction between the LOV core and the RR module. Experiments performed with radioactive ATP uncover the light-driven kinase activity. For both PST-LOV and PST-LOVΔRR much more radioactivity is incorporated when the protein is in the lit state. Furthermore, addition of the RR domain to the fully phosphorylated PST-LOVΔRR leads to a very fast transfer of radioactivity, indicating a highly efficient HK activity and a tight interaction between PST-LOVΔRR and RR, possibly facilitated by the LOV core itself.

P648

Towards a more sensitive phytoplankton?

Cristina Sobrino[1], Patrick J. Neale[2], Robert E. Moeller[3], Jason Porter[4], Jesse Phillips-Kress[2]

[1]Universidade de Vigo, Vigo, Pontevedra, Spain; [2]Smithsonian Environmental Research Center, Edgewater, Maryland, USA; [3]Miami University of Ohio, Oxford, Ohio, USA; [4]Lehigh University, Bethlehem, Pennsylvania, USA

Two different *in situ* incubations during summer 2005 and 2006 were carried out in Lake Giles (Pennsylvania, USA) to assess CO_2 effects on phytoplankton metabolism under PAR (400-750 nm) and UVR (280-400 nm). In 2005, lake water samples were aerated with 1000 ppmv CO_2 from a tank, and then incubated at 3 m depth during 6 days in 20 L polyethylene UVR transparent cubitainers. In 2006, lake samples were enriched with or without 2.5 mg/L DOC and were incubated at the surface in 2L Teflon bottles and UVR transparent bags during 6-8 days. Additionally, samples were exposed to only-PAR and PAR+UVR spectral treatments. UVR-photoinhibition was estimated from 1h polychromatic incubations with ^{14}C, using a solar simulator lamp which allowed the estimation of P-E curves and Biological Weighting Functions (BWFs) for inhibition of photosynthesis. In 2006 experiments, respiration and UVR photodegradation of DOC, increased CO_2 concentrations from 446 ppmv in the lake to maximum of 1147±26 ppmv. The results showed that higher CO_2 concentrations, either by experimental enrichment or naturally produced, promoted higher carbon fixation per chlorophyll under non-photoinhibitory light conditions. However, samples with high CO_2 concentrations were more sensitive to photoinhibitory UVR than those under lower levels of CO_2 as shown by the BWFs. The response was also related to the previous acclimation to UVR, with samples exposed to UVR being less inhibited and counteracting the effect of the high CO_2. The results support the relevance of UVR response in a future scenario of high CO_2 concentrations as well as in systems in which CO_2 concentrations are elevated by high heterotrophic or photochemical activity.

P649

Mycosporines and Mycosporine-like Amino Acids (MAAs) - an old story but ever exciting

Manfred Klisch, Donat-P. Häder

Department of Plant Ecophysiology, Institute of Botany, Friedrich-Alexander University Erlangen-Nuremberg, Erlangen, Germany

Mycosporines and mycosporine-like amino acids (MAAs) are a group of very effective naturally occurring UV-absorbing compounds distributed in a wide range of aquatic and terrestrial organisms. They have been known for decades, but they still pose many riddles. Their role as photoprotectants in cyanobacteria and eukaryotic algae is now well established, while in fungi they additionally serve regulatory purposes related to reproduction. The photoprotective function of MAAs is closely related to their localization within the organism. However, evidence on this topic is still scarce. It has been indicated that these compounds might be concentrated within the chloroplasts of eukaryotic algae. In contrast, in the dinoflagellate Gyrodinium dorsum we found that MAAs are located outside the chloroplasts, which is in agreement with the effective protection of photosynthesis from adverse effects of UV-radiation. The localization of UV-protective compounds is of special importance in small organisms such as phytoplankton or cyanobacteria. In the filamentous cyanobacterium Nostoc flagelliforme, peripheral MAAs and the sheath pigment scytonemin effectively protect the photosynthetic apparatus. Unlike scytonemin, an exclusively cyanobacterial UV-screening pigment, mycosporines and MAAs occur among organisms of remote taxonomic groups. This leads to the question of the path of their genetic dispersal. It seems likely that in the distribution of the gene cluster responsible for MAA synthesis horizontal gene transfer played a major role.

P650

Short term impact of ultraviolet radiation on photosynthesis of marine diatoms

Luis Mendía[1], Marcos Lagunas[1], E. Walter Helbling[1,2]

[1]Estación de Fotobiología Playa Unión (EFPU), Playa Unión, Rawson, Chubut, Argentina; [2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Playa Unión, Rawson, Chubut, Argentina

The aim of this study was to determine the short term impact (hours) of simulated solar radiation on four species of marine diatoms (*Thalassiosira weissflogii, Chaetoceros calcitrans, Navicula sp*. and *Cylindrotheca sp*.). Before exposure to a solar simulator (Hönle, Germany) the cultures were acclimated to “high” and “low” PAR irradiances (1230 and 33 µmol m^{-2} s^{-2}, respectively) for at least 3 days. The samples were transferred to quartz tubes and exposed to 182 W m^{-2} of PAR (400-700 nm), 70 W m^{-2} of UV-A (315-400 nm), and 1.7 W m^{-2} of UV-B (280-315 nm) for two hours under three radiation treatments (i.e. PAB, 280-700 nm; PA, 320-700 nm, and P, 400-700 nm). After exposure the cultures were allowed to recover in dim light (5 µmol m^{-2} s^{-2} of PAR) for 18 hours. Fluorescence parameters were determined during exposure and recovery using a pulse amplitude modulated fluorometer (Water PAM, Walz, Germany). Chlorophyll and absorption characteristics were also determined for each species. In all species the photosynthetic quantum yield decreased significantly during the 2 hs exposure regardless the radiation treatment or previous acclimation. The observed inhibition, however, was lower (i.e., ca 25 %) in samples in the P treatment in *T. weissflogii* and *C. calcitrans* acclimated to high irradiance. There were significant differences in the recovery of all species between pre-acclimation treatments, with samples receiving high irradiances recovering faster and having less chronic inhibition than the ones growth in low irradiance. *C. calcitrans* was the most sensitive species, hardly recovering from UVR exposure in the “low” (i.e., PAB and PA) and in the “high” (i.e., PAB) irradiance treatments. On the other hand, *T. weissflogii* had the greatest recovery rates in the “high”

irradiance treatment. All species pre-acclimated to “high” irradiance had higher concentration of carotenoids than the “low” irradiance pre-treatment. Our data suggest that the observed inhibition and recovery are not only species specific but that previous light history plays an important role in the recovery capacity of these species.

P651

Sun and shade acclimation of the photosynthetic apparatus within single leaves

Christine Ewers, Wolfgang Bilger

University of Kiel, Kiel, Germany

Higher plant leaves acclimate their photosynthetic apparatus to the prevailing irradiance. This acclimation encompasses adjustments in the size of the light harvesting complexes and the capacity of the reductive pentose phosphate cycle. A tree species very efficient in light acclimation of its leaves is beech (*Fagus sylvatica*). Sun leaves of beech show also a morphological adjustment by a permanent folding along their midrib. This causes a shading of a large area of the upper leaf surface. We studied the light acclimation of the folded and unfolded areas of such sun leaves of beech by using an imaging chlorophyll fluorometer. We detected large differences in absolute fluorescence yield (F_o and F_m) across folded leaves. Only little signs of photoinhibition (reduction of F_v/F_m) were found in the sun exposed parts of the leaves. However, when the leaves were forced to be flat and exposed to several hours natural sunlight, the previously shaded parts of the leaves developed strong photoinhibition. This was probably related to a reduced capacity for photosynthetic electron transport in these areas of the leaves. The results demonstrate the existence of strong gradients in light acclimation within single leaves and should be considered when photosyntesis is measured in leaves.

P652

High light induced ROS and their effects on photoinhibion in *Arabidopsis thaliana* mutants with reduced amount of light harvesting complexes

Milos Bartak[1], Jaroslav Lang[1], Kristyna Vecerova[1], Pavel Pospisil[2], Petr Ilik[2]

[1]Masaryk University, Institute of Experimental Biology, Department of Plant Physiology, Brno, Czech Republic; [2]Palacky University, Department of Experimental Physics, Laboratory of Biophysics, Olomouc, Czech Republic

Plant photosynthetic apparatus has several regulatory mechanisms that protect plants against negative effects of high light-induced reactive oxygen species (ROS) formed in chloroplast. In last decade, role of zeaxanthin, PsB protein, and light harvesting complexes (LHC) has been studied in npq mutants of *Arabidopsis thaliana*. These structures and underlying mechanisms of energy conversion contribute to non-photochemical quenching (NPQ) of light energy absorbed in chloroplasts. Recently, the role of amount and arrangement of LHC of photosystem II (PS II) in NPQ is investigated. The aim of our work was to quantify the effect of reduced number of LHCs on sensitivity of *Arabidopsis thaliana* to photoinhibition. In our experiments, we used mutatnts with reduced amount of LHCs of PS II, LHC proteins, main subunit of LHC II and total chlorophyll. Experimental plants exhibited either 20 % (Lhcb2-1) or 40 % (Lhcb2-12) reduction of the above components.
The Lhcb2-1, Lhcb2-12 mutants and wild type (wt) control plants of *Arabidopsis thaliana* (Columbia) were grown under controlled conditions (16/8 h d/n photoperiod, 21/19 ^{o}C d/n air temperature, relative air humidity of 55 %, photosynthetically active radiation of 140 μmol m^{-2} s^{-1}) for 42 d. Then, plants were exposed to a short-term high light stress (30 min, 2000 μmol m^{-2} s^{-1} of photosynthetically active radiation) that induced photoinhibition. Several methods were tested to quantify ROS production due to high-light treatment. The extent of photoinhibition and the rate of consequent recovery was evaluated using chlorophyll fluorescence parameters (fluorometer HFC-010, PSI, CZ). Dynamics of capacity of photochemical processes in PS II (Fv/Fm) as well as actual quantum yield of PS II (Yield PS II) was measured and the fast and slow phase of Fv/FM and Yield PS II recovery distinguished. Using the same approach, NPQ and its components qE, qI were evaluated. Immediately after photoinhibitory treatment, Fv/Fm and Yield PS II decreased to 76-78, and 76-83 % of initial values, respectively. These changed indicated structural and regulatory changes in PS II that reduced efficiency of absorbed light energy transport through PS II. Fast phase of recovery was finished after 30 min in dark followed by the slow phase (in terms of hours). After 2 h dark recovery, a 88 % recovery of Fv/Fm was achieved. Wt plants, Lhcb2-1 and Lhcb2-12 mutants exhibited different sensitivity to photoinhibition. While WT and Lhcb2-1 showed similar extent of photoinhibition and recovery of Yield PS II, the Lhcb2-2 mutants showed more pronounced photoinhibition-induced decrease in Yield PS II and no recovery within following 2 h. The results indicated that LHCs of PS II and their protein components had an important role in quenching of of excess light energy. It might be concluded that sufficient amount of LHCs II is required for an effective functioning of photoprotective mechanisms related to photosystem II. Reduction in LHC II amount may result in significant increase in sensitivity of *A. thaliana* plants to photoinhibition.

Experimental work reported in this contribution was supported by the project No. 522/06/0979 funded by the Grant Agency of the Czech Republic.

P653

Why form plants UV-B screening pigments at low temperature?

Matthias Schultze, Wolfgang Bilger

Christian-Albrechts-Universität zu Kiel, Kiel, Schleswig-Holstein, Germany

UV-B screening pigments in the epidermis reduce the UV-B radiation reaching inner tissues of plant leaves. Previous studies demonstrated that low temperature induces the biosynthesis of such pigments. What is the function of epidermal UV-B screening pigments at low temperature?
At moderate temperature plants have got effective enzymatic repair-systems to compensate damages produced by UV-B radiation not absorbed in the epidermis. However, enzymatic reactions are generally temperature-dependent and less effective at low temperature, so with decreasing temperature repair-systems of UV-B damage might become ineffective. This should lead to stronger damages by UV-B at lower temperature, if no compensation happened by an enhanced synthesis of UV-B screening pigments.
An experimental system is established where UV-B damage of photosystem II and UV-B screening in *Arabidopsis thaliana* plants is detected by chlorophyll fluorescence. The results show distinct temperature-dependencies of PS II damage and recovery after UV-B treatment. To compensate such effects at low temperature, the synthesis of epidermal UV-B screening pigments is a need!

P654

Impact of high intensity UV-B radiation on leaf chlorophyll fluorescence and content of ultraviolet-absorbing compounds of cucumber and peppermint plants

Elżbieta Skórska, Maja Wencierska

Department of Physics, Agricultural University of Szczecin, Szczecin, Poland

Among different species of crop plants a large differentiation in the susceptibility to UV-B radiation is observed. Cereals belong to more tolerant plants, whereas dicotyledonous are treated as

susceptible to this environmental factor. Relatively little data concerns wild plants, while some of them, e.g. peppermint plants are cultivated on plantations. The aim of this work was to comparative study of photosynthetic primary reactions of two species leaves subjected to high intensity UV-B radiation applied in a short period time. Plants of cucumber (*Cucumis sativus* L. cv. Dar) and peppermint (*Mentha piperita* L. cv. Asia) were grown in the controlled conditions (PPFD 200 μmol m^{-2} s^{-1}, photoperiod 12 h, 22°C/18°C, day/night). Discs (Ø 10 mm) were cut out from the leaves and placed in Petri dishes with Hoagland nutrient, then were exposed to UV-B radiation (VL-115 M, 7 W m^{-2}) for 2 h, 3.5 h and 5 h and 0 (control, in the dark), respectively. Chlorophyll fluorescence was measured by means of portable PAM-200 fluorometer (Walz, Germany); a content of ultraviolet-absorbing compounds was determined spectrophotometrically. The applied UV-B radiation even after 2 h caused a decrease of fluorescence parameters in cucumber leaves, particularly F_v/F_o, a quantum yield of PS II as well as Rfd compared to the control, and longer irradiation intensified this changes, indicating damages of photosynthetic apparatus. Peppermint leaves showed less change in the studied parameters, but visual symptoms on the leaf surface let us distinguish the irradiated and control leaves. A decrease in content of ultraviolet-absorbing compounds was observed only in cucumber leaves exposed to UV-B radiation. Generally, peppermint plants seemed to be more tolerant to the applied UV-B radiation compared to the cucumber plants.

M. Wencierska is a beneficiary receiving financing under the project "Building the region's research potential in its developmental key areas as grants for Ph. D students of Agricultural University of Szczecin developing dissertations in agriculture, fishery, food and agricultural processing and waste management" co-financed by the European Social Fund and Polish national budget in the framework of the Integrated Regional Operational Programme (IROP).

P655

Morphological and physiological changes in the UV-B irradiated plants of triticale differing in leaf wax presence

Elżbieta Skórska, Wiktor Szwarc

Department of Physics, Agricultural University of Szczecin, Szczecin, Poland

A cuticle wax layer on a leaf surface limits access of an incident light and ultraviolet radiation, increasing reflection and scattering. It was shown that some species grown under high intensity of UV-B radiation have inherently higher wax concentration. Pubescent (presence of hairs) leaves tended to be more effective in reflecting longer wavelengths than UV, whereas the glaucous leaves (presence of a thick epicuticular wax layer) demonstrated that surface waxes were very effective reflectors of both UV and longer wavelength radiation. Removal of wax can reduce reflectance. The purpose of this work was comparing the effect of UV-B radiation on the triticale traditional cultivar with the form RAH 336, genetically devoid of wax line. The plants were grown in two growth chambers at PPFD 100 μmol m^2 s^{-1}, 12 h photoperiod, 17^0C/15^0C, day/night at UV-B radiation (1.3 W m^{-2}, 4 h d^{-1}, $UV\text{-}B_{BE}$ = 3.2 kJ m^{-2} d^{-1} or not (control). The decrease of the plant height, width of leaves and dry mass after exposure of plants to UV-B radiation, comparing to the control was observed. The irradiated plants of RAH 336 were characterised by relatively stronger length decrease than plants cv. Lamberto. Total chlorophyll content was lower only in the UV-B irradiated plants of Lamberto. Both forms responded to UV-B by increasing production of flavonoids, what is one of the protective reaction activated in plants exposed to radiation. The catalase activity increased in the irradiated plants, while peroxidase activity increased only in waxless form RAH336. The UV-B had no impact on fluorescence parameters: F_v/F_o, Y and Rfd. The obtained results do not allow to state that lack of wax layer influenced on susceptibility of the studied triticale plants in early growth phase to the applied UV-B radiation. The only results that can suggest this conclusion is relative stronger effect of UV-B expressed in decrease of height.

P656

Effect of dehydration stress on delayed luminescence of plant leaves

Joonho Kang[1], Daeshik Kim[1], Daewoong Jeong[1], Sanghyun Park[2], Kwangsup Soh[2]

[1]Korea Science Academy, Busan, Republic of Korea; [2]Seoul National University, Seoul, Republic of Korea

The light radiated from all living cells and tissues is known as ultra weak photon, or biophoton. Biophoton is emitted after external light stimulates living creatures; therefore, it is called delayed luminescence (DL). The aim of this study was to investigate the effect of dehydration stress on the DL quantity of plant leaves.

In this study, samples of rose leaves, *Rosa hybrida* and *Rosa hybrida cv. Calibra*, and *Acerpalmatum* were dried under conditions of constant temperature and humidity. A photo multiplier tube (PMT) measured the delayed luminescence resulting from ultra weak photons from the living sample for three minutes. The light used for stimulation was a tungsten halogen lamp attached to a monochrometer and intensity normalizer. The samples were stimulated for 10 seconds by the light, and their delayed luminescence was measured for three min every hour during drying of the samples.

Delayed luminescence of *Rosa hybrida* increased up to 120% until two hours of sample drying passed. However, delayed luminescence decreased rapidly during the third period, i.e., the third hour, and continued to decrease gradually during the rest of the experiment. This result suggested that the dehydration stress applied to the chloroplast initially caused the quantity of DL to increase; however, as the stress accumulated in the chloroplast, it eventually lost its integrity, causing DL to decrease gradually.

To investigate any relationship of the chlorophyll in the chloroplast with the change of DL, DL measurements were performed using different colored samples of maple leaves. Green maple leaves contain chlorophyll in the chloroplast, whereas red maple leaves do not. From this set of experiments, we found that even though three maple leaves each had a different color, their DL values were not different from each other. The similarity in the quantity of DL, regardless of the color (red, or green) of plant leaves of the same species, implied that the concentration of chlorophyll in a chloroplast did not seem to affect DL. Other photosynthetic pigments in chloroplasts, such as carotenes and xanthophylls, could be possible factors that might affect DL.

P657

Effect of isotope substitution and controlled dehydration on the molecular mobility and photoinduced electron transport reactions of quinone acceptors and multiheme cytochrome c in bacterial photosynthetic reaction center

Constantine Chamorovsky, Sergei Chamorovsky, Peter Knox

Biology Faculty, Lomonosov Moscow State University, Moscow, Russia

Isotope substitution of H_2O by ^2H_2O causes an increase in the rate of dark recombination between photooxidized bacteriochlorophyll (P^+) and reduced primary quinone acceptor in *Rhodobacter sphaeroides* reaction centers (RC) at room temperature. The isotopic effect declines upon decreasing the temperature. Dehydration of RC complexes of *Ectothiorhodospira shaposhnikovii* chromatophores containing multiheme cytochrome *c* causes a decrease in the efficiency of transfer of a photomobilized electron between the primary and secondary quinone acceptors and from cytochrome to P^+. In the case of H_2O medium these effects are observed at a lower hydration than in ^2H_2O-containing medium. In the *E. shaposhnikovii* chromatophores subjected to dehydration in H_2O, the rate of electron transfer from the nearest high-potential

cytochrome heme to P^+ is virtually independent of hydration within the P/P_0 range from 0.1 to 0.5. In samples hydrated in ^2H_2O this rate is approximately 1.5 times lower than in H_2O. However, the isotopic effect of this reaction disappears upon dehydration. The intramolecular electron transfer between two high-potential hemes of cytochrome *c* in samples with ^2H_2O is inhibited within this range of P/P_0, whereas in RC samples with H_2O there is a trend toward a gradual inhibition of the interheme electron transfer with dehydration. Molecular mobility of electron carries in photosynthetic membranes was studied using the method of NMR spin-echo in samples with isotope substitution and controlled dehydration. The estimate of the mobility rate of non-water protein-containing components of photosynthetic membrane (perhaps, rotation of phospholipid molecules about longitudinal axis) at 0.2-0.3 g water/g dry weight is approximately 10^8 sec^{-1}. The experimental results are discussed in terms of the effects of isotope substitution and dehydration on relaxation processes of RC on implementation of the reactive states of RC providing electron transfer control.

This study was supported by Russian Foundation for Basic Research, project no. 06-04-48719.

P658

Ultraviolet interferential filters for the realization of sensors with spectral responses equivalent to biological action curves

M.G. Pelizzo, D. Garoli, P. Nicolosi

CNR-INFM LUXOR Laboratory and Department of Information Engineering, University of Padova, Padova, Italy

A considerable interest is growing worldwide about the photo-biological effects of the ultraviolet (UV) radiation; in fact, it has been demonstrated that UV radiation can affect both human health as well as the equilibrium of entire ecosystems. The relative effectiveness of UV radiation at a particular wavelength in producing a specific biological response can be defined by an action spectrum. Broadband radiometers characterized by having a spectral response that matches an action spectrum allow the measure of the effective irradiance associated to an ultraviolet source. Designs of innovative interference filters to be used in radiometric heads is presented. Their optimized transmission curve coupled to the spectral response of different ultraviolet photodiodes provides the match of the sensor spectral response with some selected action spectra.

P659

Photo-stability of sunscreen products in the full solar spectral range

D. Garoli[1], M.G. Pelizzo[1], P. Nicolosi[1], A. Peserico[2], M. Alaibac[2]

[1]CNR-INFM LUXOR Laboratory and Department of Information Engineering, University of Padova, Padova, Italy; [2]Unit of Dermatology, University of Padova, Padova, Italy

Studies about sunscreens photo-stability have been performed by several authors in recent years. Almost all of these are carried on by irradiating a product applied on a suitable substrate with an UV source, either a solar simulator or the natural sunlight. At the present time, at our knowledge, there are not published studies about the combined effect of UV and IR light on sunscreen photostability. In natural condition of application (seaside/summer exposure) the full spectrum spans from ultraviolet to infrared. Photostability measurements on 19 commercially sunscreen products exposed to different wavebands inside the whole UV-VIS-IR spectral range are presented; in some few cases an effect of IR radiation has been observed.

P701

Porphyrin transport in HaCaT keratinocytes after treatment with 5-aminolevulinic acid or its methyl ester derivative: effect of fumitremorgin C on intracellular porphyrin content, phototoxicity and photogenotoxicity

Claire Hill, Nicola J Traynor, Harry Moseley, William Ogilvie, James Ferguson, Julie A Woods

University of Dundee, Dundee, Scotland

Human HaCaT keratinocytes were incubated with 5-aminolevulinic acid (ALA) or its methyl-ester derivative (Me-ALA) for 24 h (uptake period). The prodrug containing medium (serum-free) was then removed, and the cells incubated for a further 24 h in drug free media (efflux period). The presence of porphyrins was confirmed by HPLC-FL. Cell-associated protoporphyrin (PpIX) was more persistent following ALA treatment, compared to Me-ALA treatment after the efflux period. The only other porphyrin consistently identified was coproporphyrin, which was found mainly in the growth medium. Pre-treating the cells with UVA before prodrug administration had a small effect in increasing cell-associated PpIX, equivalent to treating the cells with the metal-ion chelator phenanthroline. The ABCG2 transport protein inhibitor, fumitremorgin (FRG), increased the intracellular content of protoporphyrin (PpIX) during the efflux period of both prodrugs, whereas verapamil (VRA), which inhibits ABCB1 (P-glycoprotein) had minimal effect. Neither appeared to influence the concentration of coproporphyrin. The influence of the ABC transport inhibitors on intracellular PpIX content had consequential effects on markers of phototoxicity including uptake of neutral red dye, and DNA fragmentation. These data could have implications for photodynamic therapy (PDT) using porphyrin-based drugs. The results also support the recent hypothesis that changes in activity of the ABCG2 transporter could impact on the severity of erythropoeietic protoporphyria (EPP), which is characterised by a systemic accumulation of PpIX. This report demonstrates the presence of the ABCG2 transporter in HaCaT cells. Furthermore the kinetics of PpIX formation and efflux from these cells after treatment with ALA or Me-ALA mirrors that reported in skin from healthy human volunteers, indicating that this cell type could be a good model to study porphyrin transport in normal skin.

P702

Isolation and characterization of squamous carcinoma cells resistant to photodynamic treatment with methyl-aminolevulinic acid

V. Rivarola[1], F. Sanz-Rodríguez[2], A. Zamarrón[2], B. Rumie[3], I. Yslas[3], A. Blázquez[2], A. Juarranz[2]

[1]Department of Molecular Biology, Universidad Nacional de Rio Cuarto, Rio Cuarto, Argentina; [2]Universidad Autónoma de Madrid, Madrid, Spain; [3]Universidad Nacional de Rio Cuarto, Rio Cuarto, Argentina

Photodynamic therapy (PDT) using 5-aminolevulinic acid (ALA) or its derivates, are employed for the treatment of non-melanoma (NMSC) skin cancer and other skin lesions, such as actinic keratosis. However, the main problem about PDT as well as other currently used treatments against cancer is the apparition of resistant population cells. These cells would be the responsible for the recidives and metastatic process in the advanced stages of the disease. In this context the work was design to: (1) obtain resistant populations after several PDT-Me-ALA treatments from established human squamous carcinoma cell line, SCC-15 and (2) characterize molecular and morphologically the isolated resistant populations, analyzing both the organization and distribution pattern of cytoskeletal and adhesion proteins, and their relationship with survival and death pathways. For this purpose, SCC-15 cells have been exposed to several PDT treatments using methyl-aminolevulinic acid (Me-ALA) for experimental conditions that induce survival rates lower than 10 %. After two weeks of recovery, the surviving cells were harvested, replated and exposed to the next Me-ALA PDT treatment. The final population received

a total of four cycles. The cellular viability was determined using the formazan method (MTT assay) 48 h after each PDT treatment. The protoporphyrin IX production from parental and resistant cells was determined by spectrofluorometry. The morphological characterization was carried out using toluidine blue dye and Hoechst 33258 fluorochrome. The cytoskeletal organization (actin), as well as the distribution and expression of cell adhesion proteins (E-cadherin, β-catenin, β_1-integrin, vinculin), was studied using indirect immunofluorescence. Furthermore, the expresion level for the above mentioned proteins and drug resistance related mechanisms involved P-glycoprotein was analyzed by flow cytometry and western blot assays. The results obtained indicate that the present experimental model proposed could be adequate for better understanding the mechanisms for PDT-resistance with Me-ALA.

P703

Assessment of the topical photoallergy potential of METVIX cream

Douglas Brian Learn[1], Christopher Paul Sambuco[1], Paul Donald Forbes[1], Alan M. Hoberman[1], Jacques Cambrou[2], Franck Chuzel[2]

[1]The Center for Photobiology, Charles River Laboratories Discovery and Development Services, Horsham, PA, United States; [2]Galderma Research & Development, Sophia Antipolis Cedex, France

Metvix® (Methyl Aminolevulinate HCl) Cream, 160 mg/mL, is approved for photodynamic therapy (PDT) of non-hyperkeratotic actinic keratoses and superficial and non-nodular basal cell carcinoma. Metvix® Cream generates photoactive porphyrins upon exposure to red light (575-675 nm) and evokes a cytotoxic process in exposed tissues. As part of the safety assessment of Metvix®, a specific study was performed to determine the potential of Metvix® Cream to induce photoallergy (PA) in hairless albino guinea pigs. To fit with the specific mechanism of the drug action, a modified study design approach was followed. Therefore, different groups were used to assess phototoxicity and photoallergy endpoints with the red light (the therapeutic intent), UVR alone (the environmental insult) and both red light and UVR (to mimic potential environmental exposure after therapeutic exposure). During the evaluation it was needed to evaluate the maximum tolerated dose for the red light, the UV source (which contain red light) and the combination exposure) based on preceding phases of the study and the response induced by the clinical light, solar simulated light and the combination of the two source. Primary irritancy (PI), phototoxicity (PT) and contract hypersensitivity (CH) were also assessed. The dose was modified by varying the volume of Metvix® administered to 4.9 cm^2 of skin. Metvix® Cream was administered under occlusion for 2 hours and the guinea pigs were then exposed to red light (dose of 37 J/cm^2 using an Aktilite® CL128 lamp (clinical use conditions), UVR (2.25 human minimal erythema doses) or both, for PT and PA assessments. CH was assessed under identical conditions without light exposure. Administration of Metvix® Cream did not induce PI at dose volumes up to 0.6 mL. As anticipated, based on the PDT characteristics of Metvix® Cream, the dose volume was reduced to establish a minimal PT response at 0.025 ml and a NOAEL dose volume of 0.050 mL for red light and/or UVR exposure. Metvix® Cream did not induce CH at the PT NAOEL. Metvix® Cream did not induce PA at the PT NOAEL with red light, UVR or red light/UVR exposure. The skin responses observed in the PA phase were ascribed to the phototoxic response to the Metvix® Cream induced by red light, UVR or red light/UVR exposure. Body weight, body weight changes, mortality and clinical observations were unremarkable in all groups. Thus, Metvix® Cream showed no potential to induce PA in hairless albino guinea pigs.

P704

Combined effect of 5-ALA treatment with the use of NF-kB inhibitors for the survival of Glioblastoma Multiforme cells

Isabelle Coupienne, Pierre A Robe, Jacques Piette, Sébastien Bontems

University of Liege, Liege, Belgium

Glioblastoma Multiforme (GM) is the most common and the deadliest brain tumors of astrocytic origin in human adults. This cancer is a major challenge for therapists. Despite the use of surgery, chemotherapy or radiotherapy, the median survival of patients developing a type IV GM is only about 1 year. This could be explained, at least partly, by the infiltrating nature of this type of tumour in the normal brain. Several GM have also been characterized by an aberrant constitutive activation of the nuclear factor kappaB (NF-κB). This factor is largely known to play an important role in many physiological processes including inflammation, immune response, cell growth or apoptosis. It might play a critical role in the survival mechanisms induced in these GM. In various reports, the inhibition of NF-κB by the use of drugs or specific inhibitors seems to induce a somewhat important but not complete apoptosis of the GM cells. In this context, we decide to study the combined effect of NF-κB inhibitors with 5-Aminolevulinic acid (5-ALA)-based photodynamic therapy. 5-ALA is a physiological compound widely used as photosensitizer in several tumours as well as photodetectors during GM surgery. For this study, we used 3 different cells lines: U87 (type III astrocytoma) and LN18 and VC3 (both type IV). We observed and characterized by Western-blotting, Electro-mobility-Shift (EMSA) as well as by Supershift the constitutive activation of NF-κB in these three cell lines. Ours results also show that these GM cells are somewhat more resistant to 5-ALA treatment followed by its photoactivation than HeLa cells. Finally, we showed that the combined effect of 5-ALA treatment with the use of specific NF-κB inhibitors strongly decrease the survival of these GM cell lines.

P705

Activation and reorganization of skin keratinocytes and fibroblasts by photodynamic therapy with methyl-aminolevulinic acid

A. Juarranz[1], F. Sanz Rodríguez[1], A. Blázquez[1], M.C. Iglesias-de la Cruz[1], L. Bagazgoitia[2], J. Cuevas[3], S. González[2], P. Jaen[2]

[1]Department of Biology, Faculty of Sciences, Universidad Autónoma de Madrid, Madrid, Spain; [2]Dermatology Service, Hospital Ramón y Cajal, Madrid, Spain; [3]Anatomopathology Service, Hospital General de Guadalajara, Guadalajara, Spain; [4]Dermatology Service, Memorial Sloan-Kettering Cancer Center, New York, USA

Photodynamic therapy for the treatment of skin lesions, including actinic keratosis and basal cell carcinoma, is increasing. In this sense, we have analyzed the effect of the use of methyl-ALA (Me-ALA or Metvix®) in combination with red light on skin of hairless mice chronically subjected to UV light and on cell skin components: fibroblast and keratinocytes (HaCaT) in culture. Mice were exposed to chronic UV radiation for 21 weeks. Afterwards, mice were separated in groups: one group received only topical Metvix® (MAL) for 1 h, a second group received only 3 min of red light and a third group received the complete treatment (MAL-PDT). The production of protoporphyrin IX (PpIX) was confirmed by photography 1 h after topical MAL administration. Skin biopsies and photographs were taken from the dorsal areas before PDT and 21 days after treatment. Histological results indicated that MAL-PDT in the majority of the mice, significantly increased the order of the skin components in comparison with the skin of mice exposed to UV or MAL only. MAL-PDT induced: increase in the proliferation of the basal keratinocytes and of dermal fibroblasts and a decrease of mast cells. In addition, an increase in the order of extracellular components (rational distribution of elastic fibers and

increase of collagen type I) after MAL-PDT was also observed. We have also analyzed the effects of photodynamic treatments with Me-ALA that induce a cell lethality lower than 10% (evaluated by MTT test), on human fibroblasts as well as keratinocytes (HaCaT). We have observed an increase in cell proliferation as well as higher cytoskeleton organization.

P706

Evaluation of porphyrin production from 5-aminolaevulinic acid by pathogenic microbes

Ludovic Bourre[1], Francesca Giuntini[2], Ian M. Eggleston[2], Alexander J. MacRobert[1], Michael Wilson[3]

[1]National Medical Laser Center, University College of London, London, United Kingdom; [2]Department of Pharmacy and Pharmacology University of Bath, Bath, United Kingdom; [3]Division of Microbial Diseases, UCL Eastman Dental Institute, University College London, London, United Kingdom

In the present study we examined the ability of a number of microbes (*Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Candida albicans, Streptococcus pyogenes* and *Prevotella intermedia*) to produce porphyrins from 5-aminolaevulinic acid (ALA) or ALA peptide derivatives. All strains, except *S. pyogenes* and *P. intermedia*, produced porphyrins when incubated in PBS with 0.50 mM ALA for 4h. After 4h, the cultures were centrifuged and the pellet was extracted with 0.1M NH_4OH acetone solution (1:9 v/v), and the porphyrin content of the supernatant and extracted pellet were measured using fluorescence spectroscopy. The supernatant contained water soluble porphyrins that were excreted in to the medium. Gram-positive bacteria and the yeast-like fungus *C. albicans* exhibited a 622 nm emission peak for the retained intracellular porphyrins (ie from the pellet), and a 617 nm emission peak for the excreted extracellular porphyrins, corresponding to coproporphyrin III and uroporphyrin III respectively. Gram-negative bacteria exhibited a 630 nm emission peak for the intracellular porphyrins and a 615 nm emission peak for the excreted extracellular porphyrins, corresponding respectively to protoporphyrin IX and coproporphyrin III and uroporphyrin III. *P. aeruginosa* showed the highest amount of porphyrin production after incubation with ALA (arbitrary unit (a.u.): 58) then *S. epidermidis* (a.u.: 28), *S. aureus* (a.u.: 19) and *C. albicans* (a.u.: 15). No porphyrins were detected with *S. pyogenes* and no porphyrins were induced by ALA in *P. intermedia.* However *P. intermedia* showed a high content of natural endogenous porphyrins. With the aim of inducing selectivity during PDT treatment, six different ALA peptide derivatives were evaluated, with a general formula Ac-Xaa-ALA-OMe with Xaa corresponding to the D or L form of amino acid. Compounds with D and L form of Phe, Ala, Val, Leu, Pro and Trp were tested. However none of these compounds induced porphyrin production in any of the microbes tested. We are currently investigating the reasons why these microbes are not able to synthesise porphyrins from these compounds. In conclusion, the results of this study have shown that most of the microbes tested are able to produce porphyrins from exogenous 5-ALA, and should, therefore, be susceptible to lethal photosensitisation. However, in order to enhance the selectivity and the effectiveness of treatment, the development of more selective ALA derivatives is needed.

P707

Visible light inactivation of selected bacteria and fungi by modified titania

Agnieszka Jańczyk[1], Dariusz Mitoraj[1,2], Magdalena Strus[3], Wojciech Macyk[1], Piotr Bogumł Heczko[3], Horst Kisch[2], Grażyna Stochel[1]

[1]Centre for Inorganic Nanochemistry, Faculty of Chemistry, Jagiellonian University, ul. Ingardena 3, 30-060 Kraków, Poland; [2]Institute of Inorganic Chemistry, University of Erlangen-Nürnberg, Egerlandstr. 1, D-91058 Erlangen, Germany; [3]Chair of Microbiology, Jagiellonian University Medical College, ul. Czysta 18, 31-121 Kraków, Poland

Photocatalytic properties of titanium dioxide are governed by redox properties of its excited state.[1] Absorption of ultraviolet light generates an electron-hole pair. The electron from the conduction band plays a role of an efficient reducing agent whereas the concomitantly formed valence band hole is a powerful oxidizing agent. Both electrons and holes participate in formation of various reactive oxygen species (ROS; $OH^•$, $O_2^{•-}$, H_2O_2, $^1O_2$ *etc.*) capable of oxidizing the cell components: carbohydrates, lipids, proteins and nucleic acids. Irradiated titania demonstrated bactericidal effects and therefore may be used in photocatalytic disinfection procedures.[2] A possible application of this disinfection method might be useful in hospitals, microbiological laboratories, food processing plants, pharmaceutical industry, pharmacies, and wherever there is a strong requirement for clean and sterile surfaces. There are, however, very few reports on visible light induced bactericidal effect of photosensitized TiO_2.[3] We have tested visible light active titania-based photocatalysts for bacteria (*Escherichia coli, Staphylococcus aureus, Enterococcus faecalis*) and fungi (*Candida albicans, Aspergillus niger*) killing. Two materials showing photocatalytic activity (tests with photocatalytic oxidation of 4-chlorophenol under visible light irradiation) were selected. Carbon-doped titanium dioxide (C/TiO_2)[4] and TiO_2 modified with platinum(IV) chloride complexes[5] were used in suspensions or immobilized at polystyrene surfaces. In some systems a bactericidal effect induced by visible light has been observed. The detrimental effect of photocatalyst on various microorganisms decrease in the order: *E. coli* > *S. aureus* ≈ *E. faecalis* >> *C. albicans* ≈ *A. niger*.[6]

[1]Fujishima A., Rao T.N., Tryk D.A. (2000) Titanium dioxide photocatalysis. J. Photochem. Photobiol. C: Photochem. Rev. *1*, 1-21. [2]Huang Z., Maness P.-C., Blade D.M., Wolfram E.J., Smolinski A.L., Jacoby W.J. (2000) Bactericidal mode of titanium dioxide photocatalysis. J. Photochem. Photobiol. A: Chem. *130*, 163-170. [3]Yu J.C., Xie Y., Tang H.Y., Zhang L., Chan H.C., Zhao J. (2003) Visible light-assisted bactericidal effect of metalphalocyanine-sensitized titanium dioxide films. J. Photochem. Photobiol. A: Chem. *156*, 235-241. [4]Sakthivel S., Kisch H. (2003). Daylight photocatalysis by carbon-modified titanium dioxide. Angew. Chem. Int. Ed. *42*, 4908-4911. [5]Macyk W., Burgeth G., Kisch H. (2003) Photoelectrochemical properties of platinum(IV)-chloride surface modified TiO_2. Photochem. Photobiol. Sci. *2*, 322-328. [6]Mitoraj D., Jańczyk A., Strus M., Kisch H., Stochel G., Heczko P.B., Macyk, W. (2007) Visible light inactivation of bacteria and fungi by modified titanium dioxide. Photochem. Photobiol. Sci. DOI: 10.1039/b617043a.

P708

Antibacterial effect of PDT using the cationic photosensitiser

V. Negrimovsky[1], V. Mamikonyan[2], M. Balayan[2], M. Budzinskaya[2], F. Fedorov[2], M. Strakhovskaya[3], S. Shevchik[4], V. Loschenov[4], S. Kuzmin[1], G. Vorozhtsov[1]

[1]Organic Intermediates and Dyes Institute, Moscow, Russian Federation; [2]Eye Disease Research Institute RAMN, Moscow, Russian Federation; [3]M.V. Lomonosov Moscow State University, Moscow, Russian Federation; [4]General Physics Institute, Moscow, Russian Federation

Objectives: The possibility of PDT being used in an antimicrobial setting has been discussed for many years. *Staphylococcus aureus* is an increasing problem in ocular infectious diseases. Corneal trauma and contact lens wear are the most commonly accounted risk factors.

Material & Methods: To determine the efficacy of photodynamic therapy (PDT) with new polycationic photosensitiser (zink phthalocyanine bearing with eight choline groups) for treatment of corneal keratitis in a rabbit eye model. Corneal ulcer was induced in 20 rabbits using a culture of *Staphylococcus aureus* . In all animals, a corneal infiltrate developed of 5 to 8 mm diameter. After 360 hours, all eyes showed a similar corneal ulceration. Irradiation was performed after 40 min application of Cholosens in dose 2.0 mg/ ml. A diode laser («Biospec», Inc, Moscow) was used operating in the range of 675 nm. Its energy was calculated as 40 J/cm^2.

Results: In 12 days after PDT the reepithelisation of cornea with light distransparency of latter was approached in 70%.

Conclusions: These results provide evidence that PDT can produce significant regression of bacterial keratitis with no observable toxic affection to the anterior segment.

The work was carried out within the framework of scientific and technical program of Moscow Government.

P709

Targeted delivery of photosensitizing dyes via conjugation with bacterial peptide derivatives for the photodynamic therapy of bacterial infections

Ulysses Wilson Sallum, Humra Athar, Sarika Verma, Tayyaba Hasan

Wellman Center for Photomedicine, Department of Dermatology, Masschusetts General Hospital, Harvard Medical School, Boston, MA, United States

With the increased frequency of antibiotic-resistant strains of bacterial pathogens the search for effective alternative treatments grows continuously more crucial. Photodynamic therapy, (PDT), is a light activated, photochemical, emergent treatment modality for bacterial infections. The non-specific, multi-targeted, oxidative action of PDT elicits effective destruction of a myriad of infectious agents with a low probability for the development of resistant strains. Conversely, target-specificity bears its own advantages and can lower the effective concentration of drug and limit the unintended damage of host tissues. The use of photosensitizing dyes conjugated to molecules for pathogen-targeted accumulation offers the potential for lower effective drug concentrations in antibacterial PDT, while maintaining a low risk for the development of resistant strains. In this work the bactericidal effect of a phenothiazine-derivative, EtNBS, was compared with the bactericidal effect of EtNBS-cephalosporin conjugates and EtNBS-peptide conjugates across a panel of standard and clinical isolates of Gram positive and Gram negative bacterial pathogens. The implications of this work within the context of clinically applied antibacterial PDT are discussed.

P710

Photodynamic inactivation of *Allicyclobacillus acidoterrestris* with phenotiazinic dyes

Janice R. Perussi, Juliana V. Alberice, Roberta M. Maria, Emanuel Carrilho, Hidetake Imasato

Instituto de Quimica de São Carlos - Universidade de São Paulo, Sao Carlos, SP, Brazil

One of the reasons which shorten the fruit juices commercial life-time is the presence of remained microorganism contamination due to the inefficacy of fruit washing systems as well as the pasteurization of the juice, a process not efficient to destroy the spores. The previous microorganism washing out can be inadequate since primary micro flora can adhere tightly in fruit surface due interactive forces among the plant and the structure of the cell wall of the microorganisms. Besides, some non pathogenic bacteria, like *Allicyclobacillus acidoterrestris*, can be found in industrialized citric fruits juices since they are thermophilic. This kind of bacteria can cause the deterioration even of the aseptically packed juice under inadequate transport conditions, specially at very high temperatures conditions enough to activate the spores, leading to alterations on the taste and odor of the juice. Based on the facts mentioned above, there is an interest by the industry in the inactivation of this microorganism able to avoid the loose of desired organoleptic properties of the juice. The Photodynamic Inactivation can be an useful method to inactivate microorganisms such as bacteria. The principle of this procedure is the excitation by visible light of appropriated compound named as photosensitizer that leads to change of molecular oxygen to the more efficiently oxidant singlet oxygen or to formation of reactive oxygen species (ROS). All of formed reactants are able to alter the main cell functional molecules like unsaturated lipids, amino acids, nucleic acids and others. The appropriate photosensitizer is incorporated previously to irradiation. In this study, the photoinactivation of a bacteria present in the skin of some citric fruits was investigated using phenotiazinic dyes such as methylene blue (MB) and toluidine blue O (TBO). *Allicyclobacillus acidoterrestris* was isolated from the surface of orange, lemon and passion fruit and identified using Reverse Transcription (RT) polymerase chain reaction (PCR). The bacteria are grown in YGS (*yeast, glucose, starch*) medium at 45°C for 72h. The essays of photo inactivation are performed in suspension of 10^6 cells mL^{-1}, incubation times of 30 min and 24 h, light dose 27 J cm^{-2} with red LED (630nm). The live cells counting was performed through two methods: colony forming units (CFU) and by DNA microship electrophoresis. The cell counting using microchip electrophoresis (Bioanalyzer 2100, Agilent Technologies) is performed after extraction of DNA from the cells using the kit EasyDNA® followed by PCR reaction to increase the DNA concentration. The fluorescence intensity is proportional to the number of live cells. The survival index is obtained dividing the number of viable cells per culture volume by the total number of viable cells of the control culture (cells grown in the absence of sensitizer and in the dark). The results show that both used dyes are able to inactivate *A. acidoterrestris* in a concentration dependent manner but practically independent of the incubation time. For instance at 100 μg mL^{-1} of MB and TBO incubated for 24h, 95% of the bacteria were killed and for 30 min of incubation the inactivation was 90 and 85%, respectively. Comparing the cell survival index obtained by CFU with those obtained by microchip electrophoresis, the results using the CFU method was systematically lower, in other words, for all dye concentrations the viability obtained by molecular biology methods showed to be greater than by colony counting. This difference can be due to formation of some colonies from aggregated cells, underestimating the leave cells. So, the microchip electrophoresis shows to be a fast and more reliable method to study the bacteria photoinactivation. The present results using visible light plus photosensitizer are promising in order to sterilize the surface of fruits. After the optimization of several parameters such as sensitizer class, sensitizer concentration, incubation time and light dose this approach may be used for sterilization of food.

Support: FAPESP, CNPq, CAPES

P711

Photodynamic effect on *Helicobacter pylori*

M.H. El Batanouny[1,2], Rehab M. Amin[2], E.S. El Gohary[3], M.K. Ibrahium[3], M.I. Naga[1], M.S. Salama[3]

[1]Faculty of Medicine, Cairo University, Egypt; [2]NILES, Cairo University, Egypt; [3]Faculty of Science, Ain Shams University, Cairo, Egypt

Lethal photosensitization effect on *H. pylori* was studied by mixing suspensions of *H. pylori* with Toluidine Blue O (TBO) as a photosensitizer before irradiation with a Helium neon laser (632.8 nm). Zones of inhibition of bacterial growth and the number of colonies after irradiation were studied as a function of light dose and dye concentration. Ultra-structural changes and molecular genetic changes of *H. pylori* were determined after photodynamic treatment. In this study it was found that laser light or sensitizer alone did not affect bacterial viability. Irradiation with 210 J/cm² achieved 93% of bacterial killing after sensitization with TBO (100 µg/ml). The ultrastructure of *H. pylori* after PDT showed rupture of the cell wall and cytoplasmic membrane with discharge of the intracellular contents. Nine randomly amplified polymorphic DNA (RAPD) primers were used to screen genetic polymorphism in DNA of *H. pylori* after PDT. Six of them produced RAPD products while three failed to generate any product. Two bands may represent a genetic marker for *H. pylori* species or higher taxonomic position on family or order levels. It was concluded that PDT has no significant effect on the genomic DNA of the cells and the primary site of attack of PDT using TBO as a photosensitizer was mainly the cell wall and cytoplasmic membrane.

P712

Metal-phosphate coordination determines the adsorption and photodynamic activity of sulfonated metallophthalocyanines on phospholipid membranes

Alina A. Pashkovskaya[1], Elena A. Sokolenko[2], Valeri S. Sokolov[2], Elena A. Kotova[1], Yuri N. Antonenko[1]

[1]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia; [2]Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, Moscow, Russia

Photosensitized efficacy of tetrasulfonated phthalocyanines of zinc, aluminum and nickel ($ZnPcS_4$, $AlPcS_4$ and $NiPcS_4$, respectively) as studied by gramicidin channel photoinactivation was compared with adsorption of the dyes on the surface of a bilayer lipid membrane as measured by the inner field compensation method. The adsorption of the negatively charged phthalocyanines on diphytanoylphosphatidylcholine membranes led to formation of a negative boundary potential difference between the membrane/water interfaces. Good correlation was shown between the photodynamic activity and the membrane binding of the three metallophthalocyanines. $ZnPcS_4$ appeared to be the most potent of these photosensitizers, while $NiPcS_4$ was completely ineffective. All of these phthalocyanines displayed no binding and negligible gramicidin photoinactivation with membranes formed of glycerol monooleate, whereas Rose Bengal exhibited significant binding and photodynamic efficacy with glycerol monooleate membranes. Gramicidin photoinactivation in the presence of $AlPcS_4$ was inhibited by fluoride and attenuated by phosphate ions. A blue shift of the fluorescence peak position of $ZnPcS_4$ dissolved in ethanol was elicited by phosphate, similarly to fluoride, which was indicative of the coordination interaction of these ions with the central metal atom of the phthalocyanine macrocycle. This interaction was enhanced in the medium modeling the water-membrane interface. The results obtained imply that binding of tetrasulfonated metallophthalocynines to phospholipid membranes is determined primarily by metal-phosphate coordination.

P713

Dark and photohemolytic activity of some amphiphilic deuteroporphirin derivatives

Mikhail V. Malakhov[1], Galina V. Mansurova[1], Gelii V. Ponomarev[2], Andrei V. Reshetnikov[2], Alexander Ya. Potapenko[1]

[1]Russian State Medical University, Moscow, Russian Federation; [2]V.N. Orekhovich Institute of Biomedical Chemistry, Moscow, Russian Federation

Cell membranes are important targets in photodynamic therapy (PDT). For this reason, evaluation of photosensitizer (PS) membranotoxicity is a relevant problem, and PS phototoxicity test towards the erythrocytes is used for the screening of new potential PS for PDT. Photodynamic damage of erythrocytes is considered to be the side effect of PDT *in vivo*. We assessed the ability of six deuteroporphyrin derivatives (DPDs) to induce dark and photohemolysis of erythrocytes *in vitro*. Recently we have synthesized amphiphilic DPDs substituted at 2- and/or 4-position of deuteroporphyrin molecule. All of DPDs tested were able to induce dark and photohemolysis of erythrocytes. The rate of dark hemolysis varied between the various DPDs and was 1.6-7.3 times higher as compared to control sample without PS. The differences in photohemolytic efficiencies between the various DPDs were much more pronounced and varied up to four orders of magnitude. In addition, it was revealed that the number of substituents in DPD molecule plays an important role in photohemolytic efficiency of DPD. Specifically, 2- or 4-monosubstituted DPDs were found to be more effective hemolysins as compared to 2,4-disubstituted DPDs, promoting the rate of dark hemolysis 2.2-4.3-fold, and photohemolysis – 60-40000-fold. However, no correlation between dark or photohemolytic efficiencies of DPDs and distribution coefficients of these DPDs in the octanol/phosphate buffer saline (pH 7.4) system was observed. This allows us to suggest that the binding of DPD with the erythrocyte membrane lipids is not crucial factor which determines the efficiency of erythrocyte damage, and that membrane protein(s) seems to be a critical target(s) of photodynamic damage of the erythrocyte membrane. Singlet oxygen ($^1O_2$) is considered to be the main damaging factor of photodynamic treatment. We carried out the theoretical calculation of the free-path length of $^1O_2$ molecule based on the known experimental observations of oxygen diffusion in the membrane lipid phase. The calculation revealed that the free-path length of $^1O_2$ molecule is about 0.4 nm that commensurate with the size of PS molecule. This is precisely why the presence of a few substituents in PS molecule may counteract the effective photodynamic damage of the erythrocyte membrane target(s) sterically lengthening the distance between PS molecule and the membrane target(s).

This research was supported by the RFBR Grants #04-04-49626a, 07-04-01060a.

P714

***In vitro* phototoxicity study using novel phthalocyanines and incoherent light source**

Nil Saydan, Sinem Tuncer, Menaf Ayhan, Devrim Atilla, Ayse Gul Gurek, Mahmut Durmus, Vefa Ahsen

Gebze Institute of Technology, Gebze, Kocaeli, Turkey

Photodynamic therapy (PDT) is based on the using of photosensitizer and light to produce reactive oxygen species, which cause death of target cancer cells. Finding a suitable photosensitizer is crucial to improve the efficiency of PDT. Zinc phthalocyanine derivative are potential photosensitizers for PDT due to their excellent photochemical properties and high absorbtion coefficient. For PDT studies we used novel zinc phthalocyanines which were synthesized in our laboratories. HeLa cell lines were incubated in different concentrations of novel zinc phthalocyanines and illuminated using broad band incoherent light source in various energy level. Cell proliferation was determined by using MTS colorimetric assay. We observed the cells which were treated with

various concentration of phthalocyanines for 24 hours did not show dark toxicity. However, combination phthalocyanines and light showed different effects on cell proliferation of HeLa cells. The cellular uptake of phthalocyanines was observed by flow cytometry and fluorescence microscopy. Following our preliminary results we will attempt to determine the mechanism of cell death types (apoptosis or necrosis) induced by PDT in different cancer cell lines. The phthalocyanines display several in vitro characteristic that make them highly suitable for continued evaluation as PDT agents, namely dark toxicity, phototoxicity, substantial uptake by cells and favourable intracellular sites of localization.

P715

Comparative photodynamic effects of three photosensitisers in a human carcinoma cell line

Alcira Batlle[1], María Gabriela Alvarez[2], Viviana Rivarola[2], Haydée Fukuda[1,3]

[1]CIPYP - CONICET - Univ. Buenos Aires, Buenos Aires, Argentina; [2]Dept Biología Molecular FCEFQN - Univ. Rio Cuarto, Río Cuarto, Argentina; [3]Dept Quim. Biológica FCEN, UBA, Buenos Aires, Argentina

Introduction: All living cells form Haem from Aminolevulinic acid (ALA). After exogenous administration, ALA is converted into the photosensitiser (PS) Protoporphyrin IX (PPIX). PPIX is preferentially accumulated in malignant cells. After exposure to light, activated PPIX generates reactive oxygen species (ROS) leading to cell death, the so-called Photodynamic Therapy (PDT). The aim of this work has been to evaluate the photokilling activity of three PSs in Hep-2 cells.

Materials and Methods: The Hep-2 human larynx carcinoma cell line and the following PSs were used: molecular dyad porphyrinC-(60) (P-C(60)); the monocationic porphyrin derivative 5-(4-trimethyl ammoniumphenyl)-10,15,20-tris (2,4,6-trimethoxy-phenyl) porphyrin (CP) and PPIX synthesized *in situ* from ALA. The mechanism of cell death was analyzed by Hoechst -33258 and toluidine blue staining, TUNEL assay and DNA fragmentation.

Results: No dark cytotoxicity was observed with 1 mμM PC (60), 5 μM CP or 1 mM ALA along the 24 h incubation. Cell survival after irradiation with visible light was dependent on incubation time. Cells treated 24 h with P-C(60) and irradiated with 54 J/cm^2 showed 58 % of apoptosis. Instead, under these conditions, CP induced 58 % of necrosis. PPIX accumulated after incubation with 1 mM ALA for 45 h induced 40 % of apoptosis. Upon irradiation in anaerobiosis of cells incubated with P-C(60), necrosis predominated over apoptosis. Microscopy under violet-blue light, revealed that fluorescence of CP was localized in mitochondria, while ALA-induced PPIX was localized in lysosomes.

Conclusion: Photodamage in PDT is dependent on the PS, the light dose and the atmosphere conditions.

P716

Preparation and characterization of novel biofunctionalized fluorescent silica nanoparticles and their possibility for photodynamic therapy

Michihiro Nakamura[1], Masayuki Shono[2], Kazunori Ishimura[1]

[1]Department of Anatomy and Cell Biology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan; [2]Support Center for Advanced Medical Sciences, The University of Tokushima Graduate School, Tokushima, Japan

Novel biofunctionalized fluorescent silica nanoparticles made of organosilica (Organo-NPs) have been successfully synthesized by using a Stöber method. We compared the Organo-NPs to tetraethoxysilane nanoparticles (TEOS NPs). The resulting MPS NPs were well dispersed in solution and have unique surface properties such as thiol residues on the surface and reduced zeta potential compared with TEOS NPs. The Organo-NPs with fluorescent dye distributed throughout the particle were synthesized by a method that deposited fluorescent dye on the silica network via a succinimidyl ester reaction with (3-Aminopropyl) trimethoxysilane and via a maleimide reaction with organosilica. The resulting Organo-NPs with fluorescent were bright, non-aggregated in solution, and photostable. The fluorescence intensity and photostability of fluorescent Organo-NPs were sufficient for detection as a single fluorescent particle using flow cytometry and fluorescence microscopy. Protein-modified Organo-NPs were easily prepared by absorption and by maleimide coupling. They remained more dispersed as compared with TEOS NPs. We demonstrate the usefulness of Organo-NPs and their surface properties, and discuss their advantages for biological applications and possibility for photodynamic therapy.

P717

Photodynamic effect in human epithelial cells of a new methionine-porphyrin conjugate

M.A.F. Faustino[1], V. Vaz Serra[1], J.P.C. Tomé[2], M.G.P.M.S. Neves[1], A.C. Tomé[1], J.A.S. Cavaleiro[1], F. Sanz-Rodríguez[2], B. Bautista[2], A. Zamarrón[2], A. Blázquez[2], A. Juarranz[2]

[1]Department of Chemistry, University of Aveiro, Aveiro, Aveiro, Portugal; [2]Department of Biology, Universidad Autónoma de Madrid, Madrid, Madrid, Spain

The interest in porphyrin derivatives for potential application in PDT of cancer is related to their important photosensitizing properties. The possibility of modulating the photophysical and biological properties of the porphyrin macrocycle through structural modification is an important feature that has been extensively considered.[1]

In this communication we will report and discuss the interesting photosensitizing effect of a new methionine-porphyrin conjugate on tumorogenic (HeLa) and no tumorogenic (HaCat) human cells in culture. We evaluated the toxicity and phototoxicity of the conjugate, the cellular localization and death morphology induced by photodynamic treatment. The effect of photodynamic treatment on the adhesion molecule, E-cadherin and microtubules, important cell components in migration and proliferation of cancer cells, respectively, was analyzed by immunofluorescence. Under LD50 conditions, alterations in E-cadherin pattern distribution and in microtubular network of treated cells were observed. A mitotic arrest was evident 24 h after treatment and most of the metaphase cells presented altered mitotic spindle, which could also contribute to cell death by mitotic catastrophe.

[1]Bonnett, R. In *Chemical Aspects of Photodynamic Therapy*, Gordon and Breach Science Publishers: Australia, 2000.

Thanks are due to the University of Aveiro, to Fundação para a Ciência e a Tecnologia (FCT) and FEDER for funding the Organic Chemistry Research Unit. V. Vaz Serra also thanks FCT for her SFRH/BD/28122/2006 grant.

P718

Synthesis and characterization of phenothiazine- and squaraine-based photosensitizers for photodynamic therapy

Prakash Sanjeevaiah, Basel Zaitoun, Kenneth W. Olsen, David S. Crumrine

Department of Chemistry, Loyola University Chicago, Chicago, Illinois, USA

Photodynamic therapy (PDT) is an innovative approach for the treatment of cancer cells utilizing a photosensitizer, light and oxygen. Two classes of compounds have been of interest in our lab. Phenothiazine based photosensitizers (PS) such as Azure dyes are ideal candidates for PDT due to their inherent characteristics: strong absorption in the visible region of the electromagnetic spectrum, high quantum efficiency of intersystem crossing, ease of

molecular modification, and biodegradability. Commercial sources of the azure compounds pose purity issues which effect PDT efficacy and a challenge for chromatographic separation. Hence the focus shifted to use of pure phenothiazine as a starting material. Phenothiazine can be chemically modified both on the aromatic ring as well as the NH. We have synthesized a family of compounds by brominating the 3,7-positions of phenothiazine and subsequent coupling reaction using the Buchwald-Hartwig condition with variety of compounds such as pyrrole, pyrrolidine and phenothiazine. We are also interested in halogenated squaraines, a class of dyes possessing sharp intense absorption bands in the visible red to near infrared region above 600 nm with the molar absorption coefficients in the neighborhood of 500,000 $M^{-1}cm^{-1}$. PDT efficacy can be enhanced by improving the photosensitizing dyes and increasing its selectivity for tumors can enhance PDT efficacy. The second goal can be achieved by synthesizing dye-folic acid complex. The mammalian folate receptor that binds folic acid (FA) with relatively high affinity preferentially promotes endocytosis of FA. These folate receptors are highly over-expressed on many cancer cells. Attempts have been made to connect both phenothiazine and squaraine dye covalently to folic acid as a selectivity director. We have successfully made a folate acid phenothiazine conjugate using alkylation of the phenothiazine by 2-chloroethanol and esterification of the product with folic acid using DCC as a coupling agent. This product was taken up by SH-SY5Y neuroblastoma cells, which we have recently shown express excess folate receptors. The synthesis of the dye-folate complexes, investigating its photostability, characterizing its chemical and biophysical properties, its photolysis in both the presence and the absence of oxygen and determining the singlet oxygen quantum yield of the dye complex will be discussed.

P719

New porphyrin amino acid conjugates: synthesis and photodynamic effect in human epithelial cells

F. Sanz-Rodríguez[1], A. Zamarrón[1], B. Bautista[1], A. Blázquez[1], A. Juarranz[1], V. Vaz Serra[2], M.A.F. Faustino[2], J.P.C. Tomé[2], M.G.P.M.S. Neves[2], A.C. Tomé[2], J.A.S. Cavaleiro[2]

[1]Universidad Autónoma de Madrid, Madrid, Spain; [2]University of Aveiro, Department of Chemistry, 3810-193 Aveiro, Portugal

Cancer is one of today's most dreaded and mortal diseases. The development of molecules to be used in the treatment and early detection of these malignant diseases is an important issue to solve the lack of specific clinical symptoms. Photodynamic Therapy (PDT) is an emergent and promising technique for the treatment of several cancer pathologies. Among the different types of photosensitizers being used in PDT, porphyrins are the most extensively studied, due to their photophysical and biological properties.[1]

As part of a program related with the development of new compounds with potential to be used in medicine, we report here the synthesis and structural characterization of novel porphyrin derivatives conjugated with amino acid residues (*e.g.* glycine, serine, tyrosine). The photodynamic effect of the stable conjugates was evaluated on tumorogenic (HeLa) and no tumorogenic (HaCat) human cells in culture. The toxicity, phototoxicity, cellular localization of the photosensitizers, and also cytoskeletal organization and the morphology of death cells induced by the photodynamic treatment will be presented and discussed.

[1]Bonnett, R. In *Chemical Aspects of Photodynamic Therapy*, Gordon and Breach Science Publishers: Australia, 2000.

Thanks are due to the University of Aveiro, to Fundação para a Ciência e a Tecnologia (FCT) and FEDER for funding the Organic Chemistry Research Unit. V. Vaz Serra also thanks FCT for her SFRH/BD/28122/2006 grant.

P720

Development of sensitizers based on halogenated tetrapyrroles as new PDT-agents against selected melanoma and adenocarcinoma cells

Janusz M. Dąbrowski[1,2], Carlos J.P. Monteiro[3], Luis G. Arnaut[3], Mariette M. Pereira[3], Krystyna Urbańska[2], Grażyna Stochel[1]

[1]Centre for Inorganic Nanochemistry, Faculty of Chemistry, Jagiellonian University, ul. Ingardena 3, 30-060 Krakow, Poland; [2]Department of Biophysics, Faculty of Biochemistry Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387, Kraków, Poland; [3]Department of Chemistry, University of Coimbra, Rua Larga, Coimbra, Portugal

Sensitizers active in visible or preferably near infrared light are widely studied because of their possible application in the treatment of tumors. Their application as photosensitizers strongly depends on the properties of the first triplet state (T1). The triplet state formation was found to be very relevant for singlet oxygen generation which is prerequisite to promote the photodynamic effect according to type II mechanism. Good photosensitizer should combine these photophysical properties with solubility in water, photostability, a low toxicity in the dark and a tendency to accumulate in tumor cells.[1].

Considering these facts, it was tempting to examine the influence of a new water-soluble halogenated porphyrin, namely 5,10,15,20-tetrakis(2-chloro-3-sulfophenyl)porphyrin (TCPPSO3H) on S91 (mouse melanoma), SKMEL 188 (human melanoma) and MCF7 (human breast adenocarcinoma) cell lines. The results of cytotoxicity and phototoxicity as well as time dependent cellular uptake tests demonstrate its usefulness in PDT. On the other hand a major shortcoming of this porphyrin derivative is its weak absorption in the red.[2,3]

Taking into account that the penetration depth of light increases for longer wavelengths, we developed the synthesis of a family of sulpho-halogenated chlorins and bacteriochlorins.[4] In this communication we present their photophysical properties, namely absorption and fluorescence spectroscopy, triplet state lifetime, singlet molecular oxygen quantum yield and photobleaching experiments. These studies showed that TCPChlorinSO_3H and TCPBacteriochlorinSO_3H presents very promising properties to be used as PDT photosensitizers.

[1]R. Bonnett. In: Comprehensive Coordination Chemistry II, Vol. 9 2004, 945-1003. [2]J.M. Dabrowski, M.M. Pereira, L.G. Arnaut, C.J.P. Monteiro, A.F. Peixoto, A. Karocki, K. Urbańska, G. Stochel *Photochem. Photobiol.* **2007**, *83*,1-7. [3]M. Pineiro, M.M. Pereira, A.M.d'A. Rocha Gonsalves, L.G. Arnaut, S.J. Formosinho, *J. Photochem. Photobiol. A: Chem.* 2001**,** *138* , 147–157. [4]*Nouveaux derivées de porphyrine notamment chlorines at/ou bacteriochorine et leurs applications en thérapie photodynamic*, M. M. Pereira, L. G. Arnaut, S. J. Formosinho, C. J.P. Monteiro, IP Application, PCT/EP/012212 (2005).

P721

Metabolic profile and *in vivo* stability of a peptide-conjugated chlorin-type photosensitiser targeting neuropilin-1: interest of pseudopeptides

Noémie Thomas[1], Loraine Tirand[1], Denise Bechet[1], Régis Vanderesse[2], François Plénat[3], Céline Frochot[4], François Guillemin[1], Muriel Barberi-Heyob[1]

[1]Centre Alexis Vautrin-CRAN, UMR 7039 CNRS-UHP-INPL, Nancy University, Vandoeuvre-lès-Nancy, France; [2]LCPM, Groupe ENSIC, UMR 7568 CNRS-INPL, Nancy University, Nancy, France; [3]Laboratoire d'Anatomie et Cytologie Pathologiques de Brabois, CHU Nancy, Nancy University, Vandoeuvre-lès-Nancy, France; [4]DCPR-GRAPP, Groupe ENSIC, UMR 7630 CNRS-INPL, Nancy University, Nancy, France

Destruction of the neovasculature is essential for efficient tumour eradication by photodynamic therapy (PDT). Since the over-expression of Vascular Endothelial Growth Factor (VEGF)

receptors is correlated with tumour angiogenesis and growth, we conjugated a photosensitiser (5-4-carboxyphenyl)-10,15,20-triphenyl-chlorin, TPC) *via* a spacer (6-aminohexanoic acid, Ahx) to a neuropilin-1 (NRP-1) specific homing heptapeptide (ATWLPPR) targeting tumour vasculature.[1]
The intratumoural localisation of the photosensitiser, its stability and its metabolic profile have been studied on a model of nude mice xenografted with U87 human malignant glioma cells (Tirand *et al.* 2007). By fluorescence microscopy, we evidenced a selective accumulation of the conjugated photosensitiser in the endothelial cells bordering the tumour vessels compared with unconjugated photosensitiser. TPC-Ahx-ATWLPPR accumulated at high levels in the tumour and tissues of the reticuloendothelial system, particularly by liver and spleen. TPC-Ahx-ATWLPPR was stable *in vitro* in plasma for at least 24 h at 37°C. *In vivo*, the peptide moiety was progressively degraded from 2h post injection, resulting in the formation of a metabolic product, TPC-Ahx-A.[2]
In order to improve the heptapeptide stability towards proteases (Adessi and Soto 2002) and to avoid any non-selective accumulation of the metabolic product, this photosensitiser has been coupled to pseudopeptides (aTWLPPR, rpplwta and Aψ[CH_2NH]TWLPPR) on solid support. We have, for the first time, studied the affinity of these pseudopeptides towards the different $VEGF_{165}$ (isoform 165 of VEGF) receptors (NRP-1, NRP-2, Flt-1 and KDR) by competition experiments.
[1]Tirand L., Thomas N., Dodeller M., Dumas D., Frochot C., Maunit B., Guillemin F., Barberi-Heyob M. *Metabolic profile of a Peptide-conjugated chlorin-type photosensitizer targeting neuropilin-1: an in vivo and in vitro study*. Drug Metab Dispos, 2007, 35(5):806-13. [2]Tirand L., Frochot C., Vanderesse R., Thomas N., Trinquet E., Pinel S., Viriot M.L., Guillemin F., Barberi-Heyob M. *A peptide competing with VEGF165 binding on neuropilin-1 mediates targeting of a chlorin-type photosensitizer and potentiates its photodynamic activity in human endothelial cells*. J Control Release, 2006, 111(1-2):153-64. [3]Adessi C., Soto C. *Converting a peptide into a drug: strategies to improve stability and bioavailability*. Curr Med Chem, 2002, 9(9):963-78.

P722

Investigations of photosensitizer peptide conjugates for increased PDT efficacy

Alexis Lynn Reid[1], *Katie Latulippe*[2], *Roy Pottier*[2], *Eva Gudgin-Dickson*[2], *James T.C. Wojtyk*[2]
[1]*Department of Chemistry and Chemical Engineering, The Royal Military College of Canada, Kingston, Ontario, Canada;* [2]*Royal Military College, Kingston, Ontario, Canada*

Protease-Mediated Drug Delivery is a viable method for enhancing the selectivity of PDT agents as it is designed to exploit proteolytic enzymes that are over-expressed in tumour tissues. Our drug delivery constructs consist of a lipophilic photosensitizer (PS) covalently linked to a solubilizer via a polypeptide chain that is a specific substrate for the tumour-associated enzymes. Towards furthering the application of PMDD to PDT, our initial research investigated the photophysical and biochemical properties of two model conjugates of Hematoporphyrin-(HP)-IX- and Pyropheophorbide-a (Ppa) that are cleaved by chymotrypsin and trypsin, respectively, and are not specific for human tumor over-expressed enzymes. The HpIX-conjugate, consists of HpIX as the PS, linked to a tetrarginine solubilizer via a polypeptide chain containing Val-Val-Val-Phe; whereas the Ppa-conjugate is a macromolecular compound that consists of Ppa loaded on to the backbone of a poly-L-lysine chain. Results have shown that the photophysical properties of the PS are affected by the addition of the polypeptide chain and solubilizer as conjugation of these moieties to the PS does substantially reduce the singlet oxygen yield. Similarly, *in vitro* experiments have demonstrated that there is an increase in dark toxicity of the conjugated compounds compared to the parent PS; and that the macromolecular Ppa is the only compound that is capable of being internalized within the cell. These studies have been continued by generating a series of Matrix Metalloprotease-2-(MMP-2) specific PDT agents that consist of Ppa linked to four different solubilizers - $mPEGNH_2$, glucosamine, D-arginine and lysine-N-methylnicotinamide. As MMP-2 is implicated in numerous metastatic tumours, we are focussing on the viability of these drugs as novel PMDD agents when cleaved by MMP-2 through *in vitro* studies on dark toxicity, cellular localization and flow cytometry using SKOV-3 cells, which are either positive or negative for MMP-2.

P723

Chlorin e6 polyvinylpyrrolidone selectively accumulates and causes photodynamic damage in human bladder carcinoma: from chick chorioallantoic membrane model to clinical patients

William WL Chin[1], *Weber KO Lau*[2], *Paul WS Heng*[3], *Pei Li Lim*[2], *Ramaswamy Bhuvaneswari*[1], *Malini Olivo*[1]
[1]*National Cancer Centre Singapore, Singapore;* [2]*Singapore General Hospital, Singapore;* [3]*National University of Singapore, Singapore*

Introduction: Diffuse superficial transitional-cell carcinoma (TCC) refractory to standard therapies poses a clinical dilemma and photodynamic therapy (PDT) appears to be a promising treatment and palliative modality. However, PDT cause damages to the normal surrounding bladder wall and thus, prevention of these is important for bladder healing after PDT. The aim was to determine the threshold of drug and light dose for optimum selectivity and phototoxicity of the malignant bladder cells. The chick chorioallantoic membrane (CAM) was used to model tumor spheroids that resemble small residual bladder tumors prior to vascularization. The potential use of chlorin e6-polyvinylpyrrolidone (Ce6-PVP) for fluorescence detection of bladder cancer in clinical patients was also investigated.
Methods: We studied the influence of topical delivery of Ce6-PVP concentration, and irradiation parameters on the photodynamically induced damage in the CAM model. The CAM was irradiated with increasing drug (10 - 20 mM) and light dose (10 - 20 J/cm^2) and the extensive of cell death on tumor and normal CAM was evaluated by flow cytometry. Bladder cancer patients were instilled with Ce6-PVP and the resultant macroscopic and microscopic fluorescence intensity was imaged and quantified to explore possible mechanisms of penetration and distribution of the photosensitizer using fluorescence macroscopy and confocal laser scanning microscopy.
Results: We have determined the dosage of Ce6-PVP that could induce fluorescence with high sensitivity and specificity for delineating bladder cancer from the surrounding normal chorioallanotic membrane. Radiant exposures that spared normal chorioallantoic membrane were also determined. In clinical biopsies of bladder tumors, fluorescence intensity increased with the stage of disease indicating its potential use as a diagnostic marker. The depth of topical penetration into bladder tumor tissue was found to range from 100 – 200 µm.
Conclusions: We propose that the use Ce6-PVP combined with low irradiance could improve the in vivo dosimetry and optimize PDT. Our findings highlight the distinct advantageous of Ce6-PVP as a diagnostic and therapeutic agent for photodynamic therapy of bladder cancer. The potential of combined modality of tumor imaging and targeted photodynamic therapy using Ce6-PVP represents a new paradigm for bladder cancer treatment.

P724

The membrane localization of tetrapyrrolic photosensitizers as a determinant of their photodynamic efficiency. A comparison between Chlorin e6 and mTHPC

Halina Mojzisova, Stéphanie Bonneau, Christine Vever-Bizet, Daniel Brault

BioMoCeTi, CNRS UMR 7033, Univeristé Pierre et Marie Curie, Paris 6, Paris, France

The nature and the distribution of side chains around the macrocycle of tetrapyrrolic photosensitizers determine the interaction of these molecules with biological structures, in particular with membranes. Two photosensitizers are considered: chlorin e6 that bears three ionizable carboxylic chains distributed on one side of the macrocycle and mTHPC bearing four hydroxyl groups symmetrically distributed around the macrocycle.

Dioleoylphosphatidylcholine (DOPC) small unilamellar vesicles are used as simple models of lipid membranes. The three carboxylic groups of chlorin e6 interact with the polar heads of phospholipids and anchor this molecule in the vesicle hemileaflets. The transfer across the lipid membrane is extremely slow at pH 7.4. When incubated with cells, chlorin e6 localizes in spots and doesn't diffuse, at least within a short period, towards other cellular compartments, in agreement with results obtained on membrane models. On the other hand, owing to its hydrophobicity and symmetrical structure, mTHPC is likely to be localized deeper inside the bilayer. Further, the photo-induced efficiency of Ce6 is compared to that of mTHPC. In solution, these two chlorins are characterized by similar quantum yields of singlet oxygen production and methyl linoleate peroxydation. However, in a membrane environment mTHPC is 69% more efficient than Ce6 to induce methyl linoleate peroxydation. In contrast, photo-damages induced by Ce6 lead to higher permeation of membranes as indicated by leakage of carboxyfluorescein entrapped in the internal volume of liposomes. These results might be used as guidelines to design more efficient photosensitizers for photodynamic therapy and/or photochemical internalization applications.

P725

A preliminary study of photodynamic effect on matrix metalloproteinases activity in mesothelioma cell lines

Meltem Goksel Dizge, Nil Saydan, Mahmut Durmus, Ayse Gul Gurek, Vefa Ahsen

Gebze Institute of Technology, Gebze, Kocaeli, Turkey

The applications of phthalocyanines in photodynamic therapy (PDT) is important because the excited triplet state of phthalocyanine can transfer its energy to oxygen to generate excited state singlet oxygen. This species, generated in situ, is highly reactive and can be used to damage cancer cells. We developed novel phthalocyanine molecules with appropriate diamagnetic central metals, which are water soluble. In this study, the potential use of water soluble novel phthalocyanines was used for PDT. Cytotoxicity of PDT on pleural malign mesothelioma cell lines were determined by colorimetric proliferation assay. In addition, after PDT treatment determination of activity of matrix metalloproteinases (MMP's) were evaluated using gelatin zymography. MMP's are key proteinases involved in the degradation of the extracellular matrix and are identified as playing an essential role in tumor angiogenesis, growth, invasion, and metastasis. Some work shows a significant association between MMP's expression and shortened survival for patients with lung cancer. After our preliminary results *in vitro* we will focus on animal studies for understandig the effect of PDT on metastasis using novel water soluble phthalocyanines.

P726

Disorganisation of cytoskeleton and differential cell adhesion in cells resistant to photodynamic therapy

A. Casas[1], F. Sanz-Rodríguez[2], G. Di Venos[3], A. Batlle[3], J.C. Stockert[2], A. Juarranz[2]

[1]Centro de Investigaciones sobre Porfirinas y Porfirias (CIPYP)-CONICET-Hospital de Clínicas Gral. José de San Martín, Buenos Aires, Argentine; [2]Department of Biology, Faculty of Sciences, Universidad Autónoma de Madrid, Madrid, Spain; [3]Hospital de Clínicas Gral. José de San Martín, Buenos Aires, Argentine

The appearance of cells resistant to Photodynamic Therapy (PDT) is crucial for the outcome of the therapy. In the present work we analysed the differential expression and distribution of different cytoskeleton and adhesion proteins in cells resistant to 5-aminolevulinic acid (ALA)-PDT. For that purpose we employed the LM3 murine adenocarcinoma cell line and the sublines Cl4 and Cl8, isolated after successive rounds of ALA-PDT. We performed direct and indirect immunofluorescent staining, as well as Westernblot assays, to evaluate distribution the above mentioned proteins. Whereas actin stress fibers were quite well organized in the parental LM3 cells, this organisation becomes perturbed in Cl4 cells and completely loss organisation is observed in Cl8 cells. Sequential focal planes recorded from the basal to the apical cell regions show that whereas at the middle cell plane, a rim of cortical actin is conserved in Cl4 cells, the cell-substrate interface was altered. In Cl8 cells, F-actin is completely disorganised from the apical to the basal planes. In LM3 and Cl4 cells, E-cadherin and β-catenin are located at the plasma membrane connecting neighbouring cells. However, both proteins became disorganised in Cl8 cells, with interdigitations appearing in the cell to cell contacts. A nuclear distribution of β-catenin was also observed in a low percentage of Cl8 cells. In addition, whereas vinculin distribution is confined at the focal adhesion points in LM3 cells, a diffuse cytoplasmatic pattern is observed in Cl4 and more markedly in Cl8 cells. However, Westernblot assays did not show differential expression of actin, E-cadherin, vinculin or Beta-catenin. We have also evaluated adhesion to extracellular matrix proteins. Whereas the 3 lines adhered equally to fibronectin, Cl4 adhered 1.3-fold and Cl8 2-fold to fibronectin when compared to LM3. In the scratch wound healing assay, the migratory ability of the cells was: Cl8 >Cl4 >LM3, and this probably correlates with the impaired cell to cell adhesion in the resistant clones. To sum up, resistant ALA-PDT cell lines showed different pattern of cell to cell adhesion, adhesion to substrate, migration and cytoskeleton and this could be related with the different metastasic phenotype.

P727

Optimizing hypericin mediated photodynamic therapy downregulates the expression of angiogenic proteins in bladder carcinoma

Ramaswamy Bhuvaneswari[1], Gan Yap-Yik Yuen[2], Soo Khee Chee[1], Malini Olivo[1]

[1]National Cancer Centre Singapore, 11 Hospital Drive, 169610 Singapore; [2]National Institute of Education, Nanyang Technological University, Singapore

Introduction: Angiogenesis has been identified as a critical step in the progression, metastasis and regrowth of solid tumors. Hypericin, a perylenequinone derived from St John's wort is a potent photosensitizer for photodynamic therapy (PDT) due to its high triplet quantum yield and its efficient generation of singlet oxygen. PDT induces oxidative stress to achieve effective treatment and this could also lead to an endogenous angiogenic response. The aim of this study is to optimize the administration of hypericin mediated PDT to reduce the expression of angiogenic proteins in order to control tumor regrowth.

Methodology: MGH, an epithelial bladder cell line was used to inoculate tumors in 6-8 weeks old balb/c nude mice. Tumor bearing mice were treated with different treatment regime such as fractionated PDT, fractionated drug, high light dose PDT and low

light dose PDT. High light dose PDT was administered at 120 J/cm^2 and at a rate of 50 mW/cm^2 and for low light dose PDT the dosage was fixed at 50 J/cm^2 and at a rate of 41 mW/cm^2. For fractionated light protocol, high light dose PDT was administered with two rest intervals and for fractionated drug, hypericin was administered twice at 30 min and 6 h before light irradiation. Vascular and cellular targeting was also performed to study the treatment effectiveness. Treated and control tumors were monitored up to 30 days post PDT. The expression of major angiogenic proteins like VEGF, EGF, bFGF, PDGF-BB, IL-6, IL-8, TIMP-1 and 2 were analyzed using antibody arrays.
Results and Conclusions: Tumor growth in various groups were noted in the following order: control > high light dose PDT > low light dose PDT > fractionated PDT > fractionated drug. Tumors administered with fractionated drug showed better tumor response compared to the fractionated PDT group. Expression of VEGF, PDGF-BB, IL-6, IL-8, TIMP-1 and 2 was greater in fractionated PDT compared to the group treated with fractionated drug. Also angiogenic proteins were upregulated with high light dose PDT when compared to low light dose PDT. These findings clearly indicate that by modulating the drug, light and drug-light intervals of hypericin mediated PDT, the expression of angiogenic proteins can be downregulated, thus improving the overall tumor response.

P728

Characterization by MALDI-FTICR mass spectrometry of target protein potentially implied in photodynamic therapy

Benoit Maunit[1], Marc Dodeller[1], David Da Silva[1], Lina Bezdetnaya[2], François Guillemin[2], Jean François Muller[1]
[1]LSMCL-Université Paul Verlaine-Metz, Metz, France; [2]CRAN-CAV, Nancy, France

Among different treatments of cancer, Photodynamic Therapy (PDT) is an original, recent and efficient modality to reduce, even to destroy tumors. The main characteristic of a good photoensitizer for PDT purpose it to absorb at a wavelength with deeply penetrates in tissues. 5,10,15,20-tetrakis(m-hydroxyphenyl) chlorin (m-THPC, Foscan®), a second generation photosensitizer has consequently this property. These studies consist to contribute to the understanding of PDT mechanisms by working with colo HT29 cells by MALDI-FTICR Mass spectrometry. Human colon adenocarcinoma cells were deposed on a slide into multidishes to be maintained in RPMI 1640 medium supplemented with proteins and antibiotics. At the time of logarithmically growing cells, these ones were put into contact with photosensitizer Foscan® (1µg/mL) during 24 h 00. Then, they were illuminated by laser diode (λ=652 nm). Then 2D gel SDS-PAGE step is made in order to observe the protein distribution and to qualify these proteins by the intensity of their stain . Individual spots are cut out of the gel and cleaved into peptides with proteolitic enzyme (trypsin) to give a mixture of peptides. We will compare the proteins distribution of treated and untreated HT29 cells in order to highlight certain proteins implied in the PDT effect. Peptides are been identified (Peptide Mass Fingerprint) by MALDI-FTICR MS (9.4 T, Ion Spec Varian, California) to lead to the identity of the source protein after a research in NcBI-SwissProt Data Bank. The first result allowed identifying one protein which is specific to endoplasmic reticulum and which would play a role in PDT process. High resolution and high mass accuracy given by FTICR-MS seem to be major advantage for studying proteins "requested" in apoptotic pathway after Photodynamic Therapy.

P729

The effect of photodynamic therapy on mesothelioma cell lines

Murat Dogru, Sinem Tuncer, Devrim Atilla, Ayse Gul Gurek, Mahmut Durmus, Vefa Ahsen, Nil Saydan
Gebze Institute of Technology, Gebze, Kocaeli, Turkey

Despite innumerable trials of surgery, radiotherapy, and countless chemotherapeutic drugs, treatment of malignant mesothelioma is very limited by recurrent disease. Photodynamic therapy (PDT) is one of the newest and most exciting types of therapy for mesothelioma and became most valuable recently. The aim of this study is to investigate in vitro effects of phthalocyanines (Pcs) and light combination on two mesothelioma cell lines and to evaluate PDT as a possible basis for treatment. For this purpose, mesothelioma cells were incubated with increasing concentrations of phthalocyanines and in vitro uptake and visualization of phthalocyanines was evaluated by flow cytometry and fluorescence microscopy. For photodynamic therapy, cells were incubated with phthalocyanines and thereafter exposed to light for different time periods. The proliferation of mesothelioma cells after PDT were investigated using MTS colorimetric assay. Our preliminary flow cytometrical results showed 70-80% Pcs uptake in the cells. After irradiation, PDT resulted inhibition of cell proliferation of both mesothelioma cell lines. One cell line was more resistance to PDT compared to other cell line. A failure in apoptosis may contribute to the formation of a cancer and to its resistance to therapy. Our next studies will be focused on apoptosis and reletad proteins after PDT treatment. Novel therapy modalities such as PDT are desirable in curing of agressive cancer types like malignant mesotheliomas.

P730

Modulation of photodynamic injury of crayfish neuronal and glial cells by neurotrophic factors

Andrej V. Lobanov, Anatoly B. Uzdensky
South Federal University, Rostov-on-Don, Russia

Intercellular interactions are of importance for maintaining of cell survival in the complex tissue. In the nervous system neuroglial interactions are involved in survival of neurons and glial cells under stress conditions but their mechanisms are not well understood. Photodynamic therapy (PDT), which is used for treatment of brain tumours, is a strong inducer of oxidative stress. The isolated crayfish stretch receptor was used as a simple model object consisting of only two mechanoreceptor neurons (MRN) enwrapped by satellite glial cells (GC). Local MRN inactivation by an intense laser beam focused onto its body enhanced apoptosis but not necrosis of GC outside the irradiated region, which was induced by following photosensitization of the whole preparation with 10^{-7} M Photosens and 670 nm laser irradiation. Therefore, MRN body supported GC resistance to PDT-induced apoptosis. This could be due to secretion of some unidentified signaling molecules. Neurotrophic factors regulate survival of neurons and formation of neuronal networks. Exogenous mammalian neurotrophic factors NGF or GDNF but not BDNF or heregulin HRG1-β1 protected GC but not neurons from PDT-induced apoptosis. NGF also protected glial cells from PDT-induced necrosis. Heregulin HRG1-β1 precipitated PDT-induced inhibition and abolition of MRN firing. It is therefore possible that crayfish GC have receptors similar to mammalian TrkA and GFRα1/Ret receptors that recognize mNGF and GDNF, respectively. In turn, MRN has ErbB3/4-like receptors that can react to heregulin HRG1-β1. Moreover, MRN can secret NGF-like or GDNF-like neurotrophins that protect GC from PDT-induced apoptosis and GC can secret heregulin-like molecules that modulate MRN activity. As suggested in the literature, evolution of a complex central nervous system in some invertebrates like cephalopodan was associated with emergence of neurotrophic signaling. It is unknown; however, which invertebrates were the first who got neurotrophins and their receptors.

Supported by RFBR, grant 05-04-48440.

P731

Zn(II)phthalocyanine-mediated mitotic catastrophe on HeLa cells

Santiago Rello-Varona, Juan Carlos Stockert, Magdalena Cañete, Angeles Villanueva

Universidad Autónoma de Madrid, Madrid, Comunidad de Madrid, Spain

Zinc(II)-phthalocyanine (ZnPc) is one of the most promising agents in the future of Photodynamic Therapy (PDT) by its suitable photophysic and photobiological properties. PDT is capable to induce different cell death mechanisms, triggered in a dose dependent manner, including the classical necrosis and apoptosis. On the other hand, *p53* is a tumour suppressor gene that is the most commonly mutated gene in human cancer. Here we present the results of liposome-delivered ZnPc in *p53*-deficient human cervix carcinoma cell line (HeLa). ZnPc fluorescence was located near the nucleus, in a signal that corresponds to the Golgi apparatus. Sublethal ZnPc-PDT treatments induced a blockage in G2/M step of the HeLa cell cycle, as evaluated by flow cytometry. By immunofluorescence for α-tubulin, we observed a rapid metaphase arrest (6 h after light exposure). The majority of the mitotic cells showed alterations from the normal disposition of the mitotic spindle, mainly the presence of extra poles. The cell cycle arrest leads to a delayed apoptotic death (mitotic catastrophe after 48-72 h) evaluated by the TUNEL assay in flow cytometry and the cleavage of PARP protein as visualised by western blotting. In summary, ZnPc induces metaphase arrest in HeLa cells, which seems to contribute to cell death by mitotic catastrophe without p53 participation.

P732

Intracellular localisation of mTHPC and effect of photodynamic therapy in cells of the mammalian peripheral nervous system

K.E. Wright[1], A.J. MacRobert[2], J.B. Phillips[1]

[1]The Open University, Walton Hall, Biological Sciences Dept., Milton Keynes, Buckinghamshire, United Kingdom; [2]National Medical Laser Centre, University College London, London, United Kingdom

Fewer nerve-related side effects have been noted after treating head and neck cancer with photodynamic therapy (PDT) compared to conventional cancer therapy. Our aim is to investigate the biological basis for any such nerve-sparing effect. In this study the intracellular localisation and effect on cell viability of the photosensitiser meta-tetrahydroxylphenylchlorin (mTHPC) was investigated in cell culture models using peripheral nerve cells.

Primary cells from adult rat dorsal root ganglia (containing both neurons and glia) were used in these experiments. Localisation of mTHPC was detected using fluorescence and confocal microscopy. Levels of mTHPC fluorescence were quantified using digital image analysis. Immunocytochemistry with anti-β-III-tubulin and anti-S100 was used to distinguish neuronal and glial cell populations respectively. A cell-death assay using propidium iodide was used to evaluate neural cell susceptibility to PDT following incubation with mTHPC.

The results showed that mTHPC was localised in cytoplasmic regions of neurons and glia, but was not detected in neuronal axons. Necrotic cell death was detected after PDT in these neural cell types.

These results suggest that the cells of the peripheral nervous system are susceptible to PDT-mediated necrosis, but that the sparing of nerves observed during clinical PDT may be related to the heterogeneous distribution of mTHPC within neurons.

P733

LDL as delivery system of hypericin in U-87MG glioma cell line: sub-cellular distribution of hypericin and efficiency of PDT

Zuzana Nadova[1], Veronika Huntosova[1], Anton Mateasik[2], Jaromir Mikes[3], Pavol Miskovsky[1]

[1]University of P. J. Safarik/Department of Biophysics, Kosice, Slovakia; [2]International Laser Centre, Bratislava, Slovakia; [3]Institute of Biology and Ecology, P.J. Safarik University, Kosice, Slovakia

The dependence of the uptake of Hyp by human glioma U-87 MG cells on the level of expression of LDL receptors has been studied. The results obtained by fluorescence spectroscopy show that the intracellular concentration of Hyp in U-87 MG cells in the presence of LDL is proportional to the Hyp/LDL molar ratio. A special role of the LDL receptor pathway was confirmed by the increase of Hyp uptake and in the situation when number of LDL receptors on the cell surface was elevated as determined by flow cytometry. Moreover, the co-localization experiments showed the lysosomal localization of Hyp following the uptake and that the concentration of Hyp in these organelles was enhanced in the cells with elevated number of LDL receptors.

Finally a correlation between PDT effectiveness, Hyp delivery into the cells (different incubation media, activated and non-activated LDL receptors) and Hyp sub-cellular localization has been investigated.

This work was supported by the contract APVT-20-036104.

P734

The effects of increasing oxygen concentrations in PDT on cell survival and lipid peroxidation in different cancer cell lines

Astrid Hjelde, Odrun A. Gederaas, Hans E. Krokan, Alf O. Brubakk

Norwegian University of Science and Technology, Trondheim, Norway

Background: Photodynamic therapy (PDT) is based upon a chemical reaction that is limited by the availability of molecular oxygen in the target tissue. In the presence of low oxygen tension there is decreased cell sensitivity to PDT, and without oxygen, PDT has no cell-killing effect. The aim of this study was to investigate whether increased oxygen tension increased cell death compared with normoxic conditions in PDT.

Material and Methods: The effect of hyperoxia on cell survival and lipid peroxidation was investigated in two human colon carcinoma cell lines, SW480 and WiDr, and one rat bladder cell carcinoma, AY-27. The cells were incubated with 2 mM 5-aminolaevulinic acid (5-ALA) for 3.5 h at 37ºC. The cells were illuminated (1-30 min) at room temperature by blue light (435 nm) prior to hyperoxia exposure. This treatment was performed in a small temperature controlled (37ºC) hyperbaric chamber using oxygen at a pressure of 100, 200, 300 or 400 kPa.

Results: PDT performed under normoxia induced lipid peroxidation and caused a considerable decrease in cell survival. However, this decrease in cell survival was not influenced by the presence of hyperoxia. Furthermore, hyperoxic exposure (400 kPa O_2) alone caused no increase in lipid peroxidation compared to untreated control cells.

Conclusion: These data indicate that normoxic environment is sufficient to produce optimal effect of PDT. However, it still remains to find the minimum oxygen concentration below 21 kPa that is necessary to give a PDT effect. This will be investigated in different cells exposed to various degree of hypoxia.

P735

Surface-enhanced Raman and fluorescence spectroscopy of hypericin in the presence of LDL and phosphatidylcholine

Gejza Lajos[1], *Santiago Sanchez-Cortes*[2], *Daniel Jancura*[1], *Jose Vicente Garcia-Ramos*[2], *Pavol Miskovsky*[1,3]
[1]*Department of Biophysics, Safarik University, Kosice, Slovakia;* [2]*Instituto de Estructura de la Materia, CSIC, Madrid, Spain;* [3]*International Laser Center, Bratislava, Slovakia*

Surface-enhanced Raman and fluorescence spectroscopy (SERS and SEFS) were applied in the study of the interactions of photodynamically active compound hypericin (Hyp) with low density lipoproteins (LDL) and phosphatidylcholine (PCH). The SERS and SEFS spectra of Hyp in the presence of LDL and PCH were recorded using excitation line 514 nm in the interval 200-8000 cm^{-1}, which enabled us to obtain information about vibrational and fluorescence properties of Hyp from the same spectrum. The fluorescence part of the spectra informs about the state of aggregation of Hyp molecules, which is an essential information from the point of view of the biological activity of Hyp. The Raman part of the spectra provides information about vibrational properties of Hyp due to significant enhancement of the Raman bands of the molecules of Hyp, which are in the vicinity of the surface of the Ag colloid particles, used in our study as the substrate for the surface-enhanced spectroscopy. At constant concentration of Hyp, the intensity of SERS spectra of Hyp increases with the increasing of Hyp/LDL ratio. With respect to the theory of the enhancement of intensity of Raman signal in SERS it was suggested, that at low Hyp/ LDl ratios (Hyp/LDL< 30:1) the molecules of Hyp are localized in the inner part of LDL particles, which prohibits the direct contact of Hyp with the metal surface. When the number of Hyp molecules per one LDL particle is high (>100 :1), certain amount of them is localized in the phospholipid outer shell of LDL and in this situation these molecules are near the surface of colloid particles, which consequently lead to the enhancement of Raman signal of Hyp. Fluorescence part of the spectra shows a new band in the red region of the spectra (~750 nm) at high Hyp/LDL ratios (>100:1). This band is attributed to the formation of excimers of Hyp. The increasing of PCH concentration leads to the higher intensity of both fluorescence and Raman spectra of Hyp. This is due to higher solubility of Hyp in the presence of high concentration of PCH. Small, but detectable changes in the positions and relativve intensities of Raman bands of Hyp were registered upon interaction of Hyp with LDL and/or PCH in comparison with the SERS spectrum of Hyp alone.

This work was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic under the grant VEGA No. 2278, by the Slovak Research and Development Agency under the contract APVT-20-036104 and SPP Foundation (G. L.).

P736

Photochemical Internalization (PCI) with adenovirus targeted to the epidermal growth factor receptor (EGFR)

Anette Bonsted, *Håvard Kirkevold, Inger Lise Aagaard, Kristian Berg*
Rikshospitalet-Radiumhospitalet HF, The Norwegian Radium Hospital, Institute for Cancer Research, Department of Radiation Biology, Oslo, Norway

Overcoming obstacles such as low gene transfer efficiency and low vector specificity are of primary importance for the successful application of gene therapy in clinical settings. We have developed a technology, named Photochemical Internalization (PCI) for light-directed delivery of genes and macromolecules. The technology is based on photosensitizers that localize in the membranes of endocytic vesicles. A light activation of the photosensitizers induces photochemical reactions that lead to rupture of the vesicular membranes. This results in the release of endocytosed compounds (e.g. genes and macromolecules) into the cell cytosol.
Earlier, we have found that adenoviral gene delivery can be enhanced by PCI treatment. However, the first step of adenovirus transduction is binding to the coxsackie- and adenovirus receptor (CAR), which is present in many tissues and cell types. Because of the widespread expression of CAR, cell-specific adenoviral transduction appears to be limited. In this study, we used a conjugate-based approach to retarget the adenovirus to the epidermal growth factor receptor (EGFR). The adenovirus was biotinylated, reacted with the bispecific targeting protein EGF-streptavidin, purified by dialysis, and tested for its ability to transduce EFGR-positive/CAR-negative RD cells. Furthermore, the effect of PCI on transgene expression from the retargeted viruses was evaluated.
Biotinylation of the viral capsid proteins increased the virus particle size by a few millimeters and slightly reduced the negative ζ-potential of the virus. EGFR-targeted adenoviruses showed a significant increase in transduction of RD cells compared to native virus. Moreover, the percentage of transduced RD-cells increased 7-fold after PCI treatment. The PCI-induced fold-increase was similar for EGFR-targeted and native virus, indicating that adenoviral infection through CAR is not a prerequisite for obtaining photochemically enhanced transduction. Thus, PCI is a suitable strategy to combine with retargeted Ad directed against alternate (not CAR) receptors.

P737

Kinetics of the interactions of hypericin with low density lipoproteins

Luboslava Buriankova[1], *Veronika Huntosova*[1], *Diana Buzova*[1], *Daniel Jancura*[1], *Pavol Miskovsky*[1,2]
[1]*Department of Biophysics, Safarik University, Kosice, Slovakia;* [2]*International Laser Center, Bratislava, Slovakia*

By means of UV-VIS absorption and fluorescence spectroscopy we have studied the kinetics of the incorporation of hypericin (Hyp), a natural photosensitizing pigment, into low-density lipoproteins (LDL) and transfer of Hyp molecules between LDL particles. Biphasic kinetics of Hyp association with LDL was observed when solutions of Hyp and LDL were mixed together at various concentration ratios. The rapid phase of Hyp incorporation is completed within seconds, while the slow one lasts several tens of minutes. This suggests that the process of Hyp interaction with LDL is non-trivial and the existence of two types of binding sites for Hyp in LDL is proposed. The kinetics of the incorporation of Hyp into LDL particles pre-loaded with Hyp (Hyp/LDL=50:1) was also investigated. The observed decrease of Hyp fluorescence is sign of the formation of aggregates as well as of the dynamic quenching of singlet excitation state of Hyp molecules inside LDL. The characteristic time for this process is comparable with the time of the slow phase of the Hyp incorporation into LDL particles. To study the kinetics of the transfer of Hyp molecules between LDL particles, the time dependence of the fluorescence and absorbance of Hyp was followed after the mixing of the complex Hyp/LDL=200:1 with appropriate amounts of free LDL. For each final Hyp/LDL ratio the increase of the fluorescence and absorbance intensity of Hyp was observed. The half-time of this process is similar to that one of the slow phase of Hyp incorporation into LDL. All these experiments show that one phase of the incorporation of Hyp into LDL is a relatively slow process and this fact should be considered when Hyp is administered into a body.

This work was supported by the Scientific Grant Agency of the Ministry of Education of Slovak Republic under the grant VEGA No. 2278, by the Slovak Research and Development Agency under the contract APVT-20-036104.

P738

Serum albumin as carriers for phthalocyanines

Emilio Alarcón[1], Ana Maria Edwards[1], Angélica Garcìa[1], Marcelo Muñoz[1], Maureen Fajardo[1], Giulio Jori[2]
[1]Pontificia Universidad Catolica de Chile, Santiago, Metropolitana, Chile; [2]Padova University, Department of Biology, Padova Italy

Phthalocyanines are part of the group of second-generation photosensitizers for photodynamic therapy, as a consequence of their intense absorbance in the red spectral region, high photosensitizing activity and good selectivity for tumour targeting. They are also well known as efficient type II (via singlet oxygen) photosensitizers. Aggregation (especially in aqueous media) is a very common phenomenon in this family of compounds due to their large conjugated system, and this drastically decreases their fluorescent quantum yields, shortens their triplet state lifetime and reduces their photosensitizing efficiency.

We have investigated a series of phthalocyanines, with different peripheral substituents, using Zn[II]-phthalocyanine (ZnPc) as a reference compound, and we found that Zn[II]-Pc(O-Dec)8 [ZnPc(O-Dec)8] exhibited the highest singlet oxygen quantum yield. However, these compounds are highly aggregated in physiological media. In the case of ZnPc(O-Dec)8, its extremely large hydrophobicity makes very difficult its incorporation into liposomes and other delivery systems in a non-aggregated (monomeric) form.

Serum albumins are the most abundant proteins in the circulatory system. Their main function is to transport fatty acids, however they have also the capacity of binding a broad range of drugs. We have studied the incorporation of phthalocyanines (Pc) into bovine serum albumin (BSA) and human serum albumin (HSA), with the aim to obtain Pc-BSA and Pc-HSA conjugates in which the photosensitizer maintains its photosensitizing efficiency. We have determined the absorption and fluorescence spectra of the conjugates, their ability to induce post irradiation chemiluminescence (a marker of reactive oxygen species[1]), and their ability to induce phototoxicity on tumour cells when they are added to the culture media before irradiation with red light.

[1]Alarcon E, Henriquez C, Aspee A, Lissi EA. (2007) Photochem Photobiol. (in press) Chemiluminescence Associated to Singlet Oxygen Reactions with Amino Acids.

The financial support received from Fondecyt (Chile) Grant N°1040667 is acknowledged.

P739

Oligonucleotides binding constants of photosensitizer dyes and of alkylating quinones and nitroarenes with potential use in photodynamic therapy in hypoxic environments

Yisaira Diaz-Espinosa[1], Rafael Arce[2], Antonio Alegria[3]
[1]University of Puerto Rico, Rio Piedras Campus, Chemistry Graduate Program, San Juan, Puerto Rico; [2]University of Puerto Rico, Rio Piedras Campus Chemistry Graduate Program, Rio Piedras, Puerto Rico; [3]University of Puerto Rico, Humacao Campus, Chemistry Deparment, Humacao, Puerto Rico

One of the most promising anticancer therapies, discovered in the early 1900s, and still under investigation is Photodynamic Therapy. PDT uses a combination of a red laser light, a photosensiziting agent, and molecular oxygen to bring about a therapeutic effect. In PDT a Type I pathway is most efficient in hypoxic environments and high substrate concentration. This pathway involves the photoreduction or photooxidation of substrates. If the substrate is a DNA alkylating quinone or nitroarene, it could act as an alkylating species and DNA alkylation should be expected, with the consequent cell death. The objective of our work is to measure binding constants of the following photosensitizers, alkylating quinones and nitroarenes to oligonucleotides: tetrakis(1-methyl-4-pyridinio)-porphyrin tetra(p-toluenesulfonate), pyridinium zinc phthalocyanine 5-diaziridinyl-3,6-bis(carbethoxyamino)-1,4-benzoquinone, 2,5-diaziridinyl-3,6-bis(2-hydroxyethyl-amino)-1,4-benzoquinone and metroimidazole. These binding constants would allow us to determine the relative hydrophobicity and amount of the photosensitizer and quinone/nitroarene bound to oligonucleotides or to a lipid membrane. The binding constants are determined by a competition method with the well-known DNA intercalator, ethidium bromide, EB. The competition method is based on the fluorescence or absorbance titration method of EB in the absence and presence of the oligonucleotides, followed by a Scatchard analysis according to the modified equation of McGhee and von Hippel.

This work it supported by PR-AABRE Program and BDP-PRLSAMP fellowship.

P740

Full determination of PDT parameters for optimizing PDT applications

Lilian Tan Moriyama, Priscila Fernanda Campos de Menezes, José Dirceu Vollet Filho, Juliana Ferreira, Vanderlei Salvador Bagnato
Instituto de Física de São Carlos, São Carlos, SP, Brazil

Photodynamic therapy is a modality of therapy with a well established mechanism. It works quite well when applied with the required care, but it is still far from become a first choice technique. Due to the great diversity of characteristics on tumors as well as patients submitted to PDT it seems necessary to adequate parameter maintained during the PDT application that warrants the desired success. In planning PDT one needs to determine the photosensitizer concentration on the target tissue, the oxygen concentration and the light distribution. The aim of this work is to produce a combination of procedures that allow determining, in a simple way, the best possible values for the important parameters necessary to optimize PDT. Using phantoms, human anatomic parts and animal models we have developed a procedure to measure the superficial light distribution and infer through models, the approximated light distribution into the tissue. This procedure allows to determine if light dose above threshold will be acting along the whole tumor. Using fluorescence spectroscopy the amount of photosensitizer is determined as well as its spatial distribution, assuring that the desired amount of photosensitizer is present. Finally, the oxygen present in the tissue is determined using a fiber optical probe containing ruthenium salt. We shall explain the used methods and how they allow obtaining better results during the PDT application. Experiments using the proposed methodology will be presented.

Work supported by Fapesp, CNPq and CAPES.

P741

Photodynamic therapy for cancer cells using mixed wavelength LEDs

Toshiyuki Kosobe[1], Yasushi Takei[1], Xuepeng Qiu[2], Norimichi Kawashima[1], Yoshikazu Tokuoka[1]
[1]Toin University of Yokohama, Yokohama, Japan; [2]Chinese Academy of Sciences, Changchun, China

In general, PDT has been carried out using laser. However, there are still some problems in PDT using laser: the area of therapy is limited, the apparatus is expensive and large, and a special room is required for equipment. Thus, it is necessary to develop a novel and compact light source. We suggest that the use of recently developed LED, instead of lasers or halogen lamps, can solve the problems because of its low cost, simple and easy operatability, and reasonably small size.

U-937 cells were diluted with RPMI 1640 containing 10 wt% FBS to give a concentration of 5×10^5 cells/ml. Three milliliters of diluted U-937 dispersion was poured into each cell culture dish of 35 mm in diameter. The diluted U-937 dispersion was then incubated with 6µl of 10 wt% ALA physiological saline for 3 hours

at 37°C. The cells were irradiated with light from a LED for a certain period.
LEDs were employed as a light source. They emitted light of wavelengths from 400 to 700 nm, and there were specific emissive peaks in the spectrum at 455 nm for the Royal Blue LED, 470 nm for the Blue LED, 530nm for the Green LED, and 627nm for the Red LED.
PDT efficacy was in the increasing order of Red < Blue < Green < Royal Blue. Mixed wavelength LED's were more effective in PDT efficacy than that of single wavelength LEDs.

P742

Treatment of acne: the role of photodynamic therapy and micropeeling

Marcello Monti, Stefania Motta
Università degli Studi di Milano and Istituto Clinico Humanitas, Milano, Italy

Emerging problems with conventional antibiotic, retinoid and hormonal acne treatments, and their related side effects, have created a demand for safer treatments. The aim of this study was to evaluate the efficacy of a new acne treatment protocol based on aminolevulinic acid-photodynamic therapy (ALA-PDT) and micropeeling. After undergoing the appropriate washout of any previous treatment, 50 patients with mild-moderate inflammatory facial acne applied a micropeeling lotion containing glycolic and salicylic acid every night for two weeks, after which two ALA-PDT sessions were scheduled separated by a period of two weeks. A polyethylenglycol ointment containing 5% ALA was applied under occlusion for two hours and 75 J/cm^2 of red light (630 nm) was administered in eight minutes using a bifacial diode lamp, irradiance 160mW at 50 mm. One week after the PDT session, the patients resumed the topical micropeeling treatment. Each patient's acne was visually assessed by a spot count of inflammatory and non-inflammatory lesions at baseline and after one, three and six months of treatment. The acne scores of all of the patients progressively decreased in proportion to their baseline scores. The mean percentage reduction in inflammatory lesions after one, three and six months was respectively 62%, 84% and 96%. The adverse effects were transient erythema after the PDT sessions. ALA-PDT and micropeeling are effective in reducing mild-moderate acne. ALA-PDT promptly reduces inflammatory acne lesions but is scarcely efficient against comedons and microcysts, which require micropeeling. In our experience, the combination of ALA-PDT and micropeeling is more efficient than conventional therapies in cases of mild and moderate acne.This new drug-sparing treatment can be considered an advance in treatment of acne.

P743

Spectroscopic evaluation of near-IR quantum dots for cancer diagnosis

Elnaz Yaghini, Arthur Iga, Alexander M. Seifalian, Alexander J. MacRobert
Royal Free and University College Medical School, Dept. of Surgery, University College London, London, United Kingdom

Quantum dot nanocrystals are emerging as a new class of fluorescent probes for imaging of biological samples. They exhibit high fluorescence quantum yields and broad-band absorption across the visible spectral range, yet relatively narrow-band fluorescence emission. Imaging of quantum dots (QD) within tissue is possible using QDs emitting at near-IR wavelengths where tissue absorption is relatively low, and their use for localising solid tumours is now attracting considerable interest.
A spectroscopic evaluation was carried out on cadmium telluride (CdTe) quantum dots with peak emission at 800 nm and encapsulated with a new nanocomposite material based on a silsesquioxane modified poly (carbonate-urea) urethane polymer. Encapsulation with the polymer enhances the aqueous solubility and biocompatibility of the nanocrystals, and provides an opportunity to modify the surface for biomedical applications.
The fluorescence lifetime of the encapsulated versus non-encapsulated QDs was carried out using time-correlated single photon counting with excitation at 635 nm. Multiexponential lifetime decays at 800 nm were observed with the main component exhibiting a lifetime of approximately 35 ns. The presence of the polymer capping did not appear to modify the lifetime. For comparison weak fluorescence was detected from the forearm of a human volunteer under the same conditions exhibiting a considerably shorter lifetime of 2-3 ns which would enable time-gated QD detection. The photostability of the QDs was confirmed by measuring fluorescence spectra before and after exposure to a 670 nm diode laser. Cellular uptake studies in breast cancer cell lines are in progress.

P744

Possibilities for real time dosimetry for PDT

Priscila Fernanda Campos de Menezes
Instituto de Fisica de Sao Carlos-IFSC-Universidade de Sao Paulo-USP, Sao Carlos, Sao Paulo, Brazil

Effective PDT application requires adequate dosimetry. By dosimetry, one understands the application of the necessary light dose to obtain the desired necrosis of the target tissue. The achievement of the correct dosimetry depends on many parameters, which can not be easily determined. If one can resume the important parameters to better quantify the photodynamic reaction, the dosimetry would become practical and easy. The occurrence of a photodynamic reaction leads to tissue necrosis. The question is how to evaluate and quantify the photodynamic reaction? To do that, we have used the singlet oxygen reaction as an indicative of PDT effect. Under light and in the presence of oxygen, photosensitizers molecules undergo phototransformation, which is the result of oxidative reaction from singlet specie produced. By measuring *in vivo* phototransformation we are able to quantity the photodynamic reaction and therefore associate the light dose to achieve the desired necrosis. In this work it was established a correlation between the degradation *in vivo* of Photogem with the depth and width of necrosis produced in normal liver of rats. The same irradiation dose (0-450 J/cm^2) was used in two conditions; one maintaining the irradiation intensity constant at 250 mW/cm^2 and applying different times and another using different irradiation intensity at constant time (840 s). Our results involving *in vivo* and *in vitro* studies indicate the possibility for a real time dosimetry during PDT, where maintaining the variation of photosensitizer fluorescence one can feedback the procedure to achieve the final goal. During this presentation several experiments comparing this work will be discussed.
Work supported by FAPESP/CNPq/Capes.

P745

Early diagnosis of cancers by fluorescence imaging of cytologic slides

Karine Steenkeste[1], Ariane Deniset[1], Sandrine Lécart[2], Sandrine Lévêque Fort[1], Pascal Eschwege[3], Sophie Ferlicot[4], Michèle Fabre[4], Basile Maldant[5], Marie-Pierre Fontaine-Aupart[1]
[1]Laboratoire de Photophysique Moléculaire, UPR 3361 CNRS, Université Paris Sud Orsay, Orsay, 91405, France; [2]Centre de Photonique Biomédicale (CPBM), Université Paris-Sud, Orsay, France; [3]Service d'Urologie, Hôpital Bicêtre, Université Paris-Sud, Le Kremlin Bicêtre, France; [4]Service d'Anatomie et Cytologie Pathologiques, Hôpital de Bicêtre, Le Kremlin Bicêtre, France; [5]Laboratoire d'Anatomie Cytologie Pathologiques, Bièvres, France

An early diagnosis of malignant cells is essential to increase the therapy success and the patient survival. Thus, the enhancement of early cancer diagnosis and treatment has involved the development

of new methods to identify such malignant cells. To this aim, we have an interest in fluorescence imaging methods for *ex vivo* diagnosis of bladder, uterus and pancreas cancers. In a first step, the current standard to diagnose the presence of tumoral cells and to follow-up patients during and after treatments is based on morphological observations of the cell structure by transmission microscopy. However, the method is of limited value because of the operator dependency and its low sensitivity especially for cancerous lesions named "low grade" or for atypical cells for which it exists a high proportion of false negative.
In this work, we present a detailed study of the fluorescence properties of classical Papanicolaou-stained urothelial cytological slides from patients or from cell lines to investigate metabolic changes in normal, tumoral and atypical cells. The fluorescence signature of each cell type in terms of fluorescence intensity, spectra and lifetime were measured in the spectral range 400-800 nm. In these conditions, the fluorescence emission domains of endogenous fluorophores (NAD(P)H, flavoproteins, and porphyrins) and of Papanicolaou stains were covered.
The results thus obtained have pointed out a difference in the fluorescence localization between activated cells (among them, tumoral cells) characterized by a perimembranar fluorescence and resting cells for which the fluorescence was exclusively intracellular. Spectral and lifetime measurements ascertain that this specific fluorescence signature was not correlated to the emission of endogeneous fluorophores but to the EA 50 Papanicolaou stain. These present results demonstrate that the use of single-cell endofluorescence emission of Papanicolaou-stained urothelial cytological slides can allow an early *ex vivo* diagnosis of low-grade bladder cancers. An equivalent approach is developped for cervival and pancreatic cancers.

P746

The photodynamic action *vs* catechol

Martina Bancirova, Jana Jarošová, Veronika Borská
Palacký University, Department of Physical Chemistry, Olomouc, Czech Republic

Anti-oxidant or pro-oxidant effect during the photodynamic action was studied. Catechol is a chemical compound originally isolated from a type of mimosa tree. Small amounts of catechol occur naturally in fruits and vegetables, along with the enzyme polyphenol oxidase. Upon exposure to air (as when a potato or apple is cut), the colorless catechol oxidizes to reddish-brown benzoquinone. This accounts for the browning of cut fruit and vegetables. Catechols are typical antioxidants which can protect organism against the oxidative damage (as the scavengers of free radicals due to their catechol group).
The chemiluminescence can not only be used for study of ROS but also for study of the antioxidant and pro-oxidant properties of different kinds of compounds. This method is TAC (the total antioxidant capacity) determination by measurement of the length of inhibition time of luminol radical dependent chemiluminescence, assuming that it is directly proportional to the total antioxidant potential. The source of ROS was the system of hydrogen peroxide decomposition (e.g. by enzymes or metal ions). The reaction system was affected by the concentration range, temperature and lifetime of reaction and light. The comparison with the biological system with the photosensitizers was achieved on *Paramecium caudatum.*

P747

Products of photosensitizer photooxidation are responsible for systemic immunosuppression

Alla A. Kyagova[1], Elena A. Kozhinova[1], Lyudmila A. Kozir[1], Galina V. Mansurova[1], Zoya I. Moshnina[2], Gelii V. Ponomarev[3], Alexander Ya. Potapenko[1]
[1]Russian State Medical University, Moscow, Russian Federation; [2]Psorias Centre, Moscow, Russian Federation; [3]V.N. Orekhovich Institute of Biomedical Chemistry, Moscow, Russian Federation

Application of photosensitizers in medicine [Psoralen+UVA-therapy (PUVA) or Photodynamic Therapy (PDT)] is frequently accompanied by induction of systemic immunosuppression. We found that this effect can be initiated by products of photosensitizer photooxidation. Oral administration of photooxidized psoralen (Ps), 8-methoxypsoralen (8-MOP) and 5-methoxypsoralen (5-MOP) induced suppression of Contact Hypersensitivity (CHS) reaction to 2,4-dinitrofluorobenzen and oxazolone in mice. Immunosuppressive efficiency decreased in a row: 5-MOP=8-MOP>Ps. All photooxidized psoralens induced suppression of the CHS in a dose dependent manner. Intravenous injection of preirradiated solution of protoporphyrin IX (PPIX) or merocyanine 540 (MC540) to mice resulted in dose-dependent suppression of the CHS. Solutions of photodecomposed PPIX contained chlorin-type products, namely, two isomers of photoprotoporphyrin (pPP) as main photoproducts exerting equal immunosuppressive activity. Suppression of the CHS induced by pPP, preirradiated Ps or MC540 was adoptively transferable and was associated with generation of cells with suppressive functions. Suppression induced by preirradiated Ps was antigen-specific, in contrast to that effects of preirradiated MC540 or PPIX were non-specific. Our results strongly indicate that induction of systemic immunosuppression by PDT or PUVA may proceed through photooxidation of photosensitizers and generation of photoproducts, which can affect T cell immunity.

This research was supported by the RFBR Grants #04-04-49626a, 07-04-01060a.

P748

Fluorescence imaging for demarcation of Basal Cell Carcinoma tumor borders

Claes D. Enk[1], Joseph Alcalay[2], Clemens Fritsch[3], Thomas Ruzicka[4], Ronen Alkalay[1]
[1]Dept. Dermatology, Hadassah Medical Center, Jerusalem, Israel; [2]Mohs Surgery Unit, Assuta Medical Center, Tel Aviv, Israel; [3]Dept. Dermatology, University of Dusseldorf, Munich, Germany; [4]Dept. Dermatology, University of Munich, Munich, Germany

Background: Basal Cell Carcinoma (BCC) is the most common form of nonmelanoma skin cancer worldwide. BCCs are slow-growing, locally invasive tumors that rarely metastasize but can cause extensive morbidity through local tissue destruction. Recurrence is often the consequence of incomplete removal of the cancer tissue. Mohs' micrographic surgery is considered the most effective treatment modality for BCC with a recurrence rate of less than 5 %, however, the technique requires specialized training and is labor-intensive and costly. Fluorescence imaging by topical application of a tumor-localizing agent such as methyl 5-aminolevulinic acid (MAL), resulting in buildup of photosensitizing porphyrin IX (PpIX) that can be visualized by Woods' light fluorescence, might serve as a quick and simple "bed-side" technique for demarcation of BCC tumor borders prior to conventional surgical excision.
Objective: To test the reliability of MAL-induced porphyrin fluorescence tumor demarcation by comparison with the tumor borders determined by Mohs' surgery.
Methods: Twenty eight patients with facial BCCs (17 nodular, 3 superficial, 1 morphea type, 3 ulcerated and 4 recurrent tumors) scheduled for Mohs' surgery were recruited for the study. The night before the surgical procedure, crusts were gently removed and an approximately 1 mm thick layer of a cream containing 16 % MAL

(Metvix[R]) was applied to the tumor area as well as to the surrounding skin and covered by a transparent occlusive dressing. The following morning (10-17 hours after Metvix[R] application), the dressing was removed, and the lesion size was determined with a caliber by measuring the largest perpendicular diameters under natural (*clinical size*) and Woods' (*fluorescence size*) illumination. The patients then underwent the scheduled Mohs' surgical procedure, and the tumor size (*Mohs' size*) was determined when reaching the tumor free margins
Results: Median difference between *Mohs' size* and *clinical size* was 42.9 mm^2 and between *Mohs' size* and *fluorescence size* was 25.9 mm^2. The reliability of estimates of *fluorescence size* and *clinical size*, respectively, compared to *Mohs' size* was determined by the interclass correlation coefficient: 0.591 (*Mohs' versus clinical*) and 0.686 (*Mohs' versus fluorescence*).
Conclusions: Though MAL-induced porphyrin fluorescence imaging tends to underestimate the true tumor size, the procedure is more accurate than tumor border demarcation by clinical assessment. However, tumor demarcation by fluorescence imaging in the present form combined with excision surgery cannot replace Mohs' procedure in the treatment of BCC.

P749

Induced phototoxicity of cell culture media

Thierry Patrice, David Olivier, Samuel Douillard
Cancer Photobiology, HGRL, Nantes, France

Photochemotherapy is based on the use of photosensitizing chemicals that in combination with light act via type I or type II mechanisms under light exposure. A recurrent discussion occurs to identify whether vascular structures or cancer cells are the main PDT target for type II reactions. Recently some sensitizers had been specially designed and successfully used for the photo treatment of abnormal vessels during degenerative diseases. In such applications it is generally considered that vessel occlusion occurs through coagulation processes secondary to endothelial cell damages. In order to verify whether the photochemical reactions occurring in the vessel lumen thus out of EC could lead to a certain level of toxicity we exposed regular cell culture media RPMI-FCS incubated with sensitizers (methyl tetra hydroxyphenyl chlorine (m-THPC, Foscan®) or benzoporphyrine derivative (BPD, Visudyne®) but also rose bengal (RB) a sensitizer known to be a well characterized $^1O_2$ producer) to light before transferring them to untreated chemoresistant F 98 cells (DT 20h) or C6 murine glioma cells (DT 16h), HT 29 human colonic cancer cells (DT 25h), L 1210 a lymphoblastic murine leukemia (DT 8h) . Cell survival was monitored after 0 to 120 minutes of contact. We observed a decrease of cell survival varying according to cell type, cell doubling time and the sensitizer used to treat the cell medium. Cell growth inhibition was sensitizer and light dose related.
Although this cell growth decrease measured after 24 hours had been shown to be proportional to the sensitizer or the light dose given, differences with intensities of cells growth decreases were found for one given sensitizer. BPD treated media appeared to be the most inhibiting (80%), then RB (50%) and m-THPC (20%). However for m-THPC cell survival inhibition was found more marked (80%) 48 hours after treatment when for BPD or RB it was on the contrary less marked than after 24 hours, 20% and 35% respectively. This led us to replace only a percentage of the native media by photo treated media. In these conditions a decrease in cell survival had been observed proportional to the amount of photo treated cell media transferred. At this step culture media and sera of several origins had been assayed using RB (10ug/ml) as sensitizer and 514 nm, 10J/sqcm as light delivered. The above described cell growth decrease was found to be inversely proportional to the cell doubling time and thus whatever the parameters of photo treatment of media before addition to cells.

P750

Phototoxicity is not associated with Rose Bengal-photosensitized tissue bonding

Min Yao, Kenneth Bujold, Robert Redmond, Irene Kochevar
Wellman Center for Photomedicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States

We have recently developed a novel technique called photochemical tissue bonding (PTB) for sealing surgical wounds, repairing nerves, blood vessels and other structures and for placing grafts. Rose Bengal (RB) is applied to the wound surfaces, which are then placed in contact and irradiated for 1-3 min with green laser light. Photocrosslinking reactions between proteins on the surfaces form an immediate water-tight seal without thermal damage. RB is well-known as a phototoxic dye to cultured cells, but enhanced inflammation, compared to sutures, is not associated with PTB. We sought to understand this unexpected (but desirable) result. Cell death was assessed in porcine skin after PTB. Full thickness 2-cm incisions in fresh porcine skin were secured with subcutaneous sutures before treatment with 0.1% RB and 100 J/cm^2 532 nm light. Controls received no light or no RB. Tissue was placed in organ culture for 24 h. Dead cells (pyknotic nuclei, eosinophilic cytoplasm) were the same ($p > 0.05$) in the PTB-treated and control epidermis and dermis. Fluorescence confocal microscopy of RB-stained incisions revealed that RB diffused only ~100 μm from the dermal incision surface, suggesting that RB binds strongly to extracellular matrix. To determine whether RB phototoxicity is prevented by the matrix environment of the cells, we compared RB phototoxicity to dermal fibroblasts (FB) grown in a collagen matrix to that for FB grown in tissue culture. The intracellular concentration of RB was determined for cells grown under both conditions. At the same intracellular concentration, RB was at least 100-fold less phototoxic to FB grown in collagen gel. These results indicate that the tissue environment enhances the survival of FB after RB plus light treatment and suggest that RB phototoxicity is not a serious concern for medical applications of PTB.

P751

Photochemical reactions of bilirubin and folic acid in different solutions

A. Vorobey[1], V. Plavsky[2], P. Vorobey[3], A.H. Steindal[4], J. Moan[4,5]
[1]Institute of Biophysics and Cell Engineering, National Academy of Sciences of Belarus, Minsk, Belarus; [2]Institute of Physics, National Academy of Sciences of Belarus, Minsk, Belarus; [3]International Sakharov Environmental University, Minsk, Belarus; [4]Institute for Cancer Research, Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway; [5]Department of Physics, University of Oslo, Oslo, Norway

Phototherapy is the most common treatment of jaundice for infants. Its aim is to convert the excess bilirubin in blood to water-soluble photoproducts which can be excreted. It is believed that phototherapy of jaundice may be done without any serious side effects. However, some bilirubin photoproducts possess a photodynamic activity. This may cause photodamages to life-important molecules, for example, folates, which are highly sensitive to $^1O_2$. We investigated the mutual influence of bilirubin and folic acid (FA) in photochemical reactions. Water-DMSO (20:1) solutions of bilirubin ($2 \cdot 10^{-5}$ M) and FA ($2 \cdot 10^{-5}$ M) without and in the presence of human serum albumin (HSA, $2 \cdot 10^{-5}$ M) were exposed to blue light (luminescent lamps, λ_{max}=420 nm, 50 W/m^2). Oxygen-depleted solutions were obtained by nitrogen bubbling. Photochemical transformations were studied with absorption and fluorescent spectroscopy. Blue light exposure (10–30 min) of bilirubin solution cause drastic decrease of absorbance at 400–500 nm and an increase of absorbance at 520–700 nm. In the presence of FA the rate of bilirubin phototransformation increased, but less photoproducts with absorbance at 520–700 nm were formed. Oxygen-depletion of FA/bilirubin solution led to slower

bilirubin phototransformation. Addition of HSA in bilirubin solution caused decrease in the rate of bilirubin phototransformation, but more products with absorbance at 520–700 nm were formed. In FA/bilirubin solution with HSA the rate of bilirubin phototransformation was lower than in solution without HSA. Oxygen-depletion of HSA-containing FA/bilirubin solutions had low effect on the rate of bilirubin phototransformation. Under conditions used, we registered the accumulation of fluorescent FA photoproducts, in particular, 6-formil-pterin (FPT), that absorb light till 420 nm. The presence of bilirubin in solution has no effect on this process. Thus, bilirubin photoproducts do not sensitize FA photodegradation. At the same time, as it was mentioned above, FA caused an increase in the rate of bilirubin phototransformation. We believe that this phenomenon is related to generation of $^1O_2$ by FA photoproducts. This is confirmed by the decrease of the effect in oxygen-depleted solutions and ability of FPT to photosensitize bilirubin degradation.

P752

Differentiation of curcumin phototoxicity in cells and bacteria by change in pharmaceutical preparation

Tone Haukvik[1,2], Ellen M. Bruzell[2], Inger Sofie Dragland[2], Else Morisbak[2], Hanne Hjorth Tønnesen[1]

[1]School of Pharmacy, University of Oslo, Oslo, Norway; [2]Scandinavian Institute of Dental Materials (NIOM), Haslum, Norway

Photodynamic therapy (PDT) offers a promising alternative to antibiotic treatment against oral infections and may act as an effective anticancer therapy against oral lesions. Curcumin (CU) has potential as a photosensitizer for oral applications. CU is a natural, yellow compound with absorption maximum at 430 nm. CU is practically insoluble in water at acidic or neutral pH. At pH above neutral the solubility increases, but the compound then undergoes rapid hydrolytic degradiation. Therefore, it is a challenge to make an aqueous CU preparation with an acceptable solubility and stability at physiological pH. We have previously shown that low concentrations of CU (≤0.7 µM) in DMSO, micelles, cyclodextrin (CD) and liposomes(LP) have phototoxic effect on non-cancerous rat submandibular gland (oral) cells, (SM 10-12), in combination with blue light (em. max 490 nm) at a low light dose (≤6 J/ cm^2) emitted from a halogen lamp. Cell death was determined by staining/fluorescence microscopy. The SM 10-12 cells were most susceptible to CU in the CD and LP preparations. We have further demonstrated phototoxic effects to oral bacteria of CU in selected aqueous preparations. Enterococcus Faecalis was exposed to 2.5 µM CU in the same preparations as in the SM 10-12 cell experiment. After 30 min incubation the bacteria were irradiated with fluorescent tubes emitting blue light (em. max 430 nm). The irradiance was $17 mW/cm^2$ and the light dose $0.5 J/cm^2$, less than 10 times lower than in the SM 10-12 experiment. The bacteria survival was calculated as a percentage compared to controls. Different post-irradiation incubation times were tested. The DMSO and micelle preparations were the most efficient vehicle for CU to induce killing of E. Faecalis (<0.5% survival). CU possesses native fluorescence and the uptake of CU in both bacteria and SM 10-12 cells was examined by fluorescence microscopy. As a conclusion, the phototoxic effect was dependent on CU concentration, light dose, post-irradiation incubation times (tested for bacteria only) and most importantly, the type of preparation. There are differences in sensitivity between bacteria and cells to PDT with CU as the sensitizing compound as a function of preparation properties. These results indicate that it may be possible to direct CU to either cells or bacteria by use of an appropriate pharmaceutical preparation and thereby to achieve a targeting effect. Ongoing work includes CU phototoxicity studies on other bacteria species, grown as single colonies and in biofilms, and the effects of other aqueous preparations of CU.

A

Aagaard Inger Lise ... P736
Aalerud Tommy Nakken ... P629
Ablamsky Danielle ... IL160
Agostinis Patrizia ... OC436; IL252; IL138
Ahmad Margaret ... P646
Ahsen Vefa ... P729; P725; P714
Aigelsreiter Ariane ... OC307
AlAdly Amira Abdou ... OC236
Alaibac M. ... OC659; OC358
Alarcón Emilio ... P738
Alberice Juliana V. ... P710
Alcalay Joseph ... P748
Alegria Antonio ... P739
Alkalay Ronen ... P748
Allan Donald ... P621; OC320; OC207
Almeida M.A. ... P642
Alsherbini Alsayed Abdelmajid ... P641
Alvarez María Gabriela ... P715
Amelink Arjen ... IL504; OC218
Amin Rehab M. ... P711
Ananthaswamy Honnavara N. ... OC435
Andersson-Engels Stefan ... OC134
Andreu Inmaculada ... P637
Angres Brigitte ... IL128
Antonenko Yuri N. ... P712
Aphalo Pedro Jose ... OC364
Arce Rafael ... P739; P638; P631; IL333
Arnaut Luis G. ... P720
Arnim Christine v. ... IL130
Arnoldi F. G. C. ... IL157
Arum Carl-Jørgen ... OC338
Ashmarov V.V. ... OC212
Asselin Claude ... OC308
Athar Humra ... P709; OC429; IL238; IL137
Atif Muhammad ... OC509
Atilla Devrim ... P729; P714
Axelsson Johan ... OC134
Ayhan Menaf ... P714
Aymon Daniela ... OC506

B

Bagazgoitia L. ... P705
Bagnato Vanderlei Salvador ... P740
Bais Alkiviadis ... P622; OC321
Bakioui Sawsane ... OC353
Balayan M. ... P708
Balmer Paul ... P623
Bancirova Martina ... P746; P644
Barberi-Heyob Muriel ... P721; IL240
Barge Jérôme ... OC508
Baron Elma D. ... OC142
Bartak Milos ... P652
Bassukas Ioannis ... OC507
Bastien Nathalie ... OC308
Batlle Alcira ... P726; P715; OC113
Batschauer Alfred ... IL518
Bautista B. ... P719; P717
Bayer Anette ... P612
Becherer Alexander ... OC416
Bechet Denise ... P721; IL240
Becker Wolfgang ... IL127
Bednarski Patrick J ... OC224
Behrend Kristina ... OC437
Belaidi Jean-Philippe ... P608; IL330; IL303
Belaubre Françoise ... OC353
Bella Zsolt ... IL413
Belmadoui Noureddine ... P633; P604
Bendsoe Niels ... OC134
Benjamin Cara L. ... OC435
Berces A. ... P611
Berg Kristian ... P736; OC345; IL241; OC143
Bergh Hubert van den ... OC508
Berghoff Bork ... OC367
Bergmann Axel ... IL127
Berndt Alex ... P646
Berner Aasmund ... IL110
Bernerd Françoise ... OC150
Bernstein Shoshana ... IL311
Berrahmoune Saoussen ... OC337
Berry Jacqueline L ... P621; OC320; OC207
Besaratinia Ahmad ... IL403
Bezdetnaya Lina ... P728; OC337; OC141; OC112
Bhuvaneswari Ramaswamy ... P727; P723
Bianchini Paolo ... IL258
Bibby Thomas S. ... IL154
Bichinho Gerson Linck ... P615
Bilger Wolfgang ... P653; P651; IL439; IL231
Bilski Piotr J. ... IL513
Birch-Machin Mark ... OC226
Bishop June E ... OC228
Biszczuk Julita ... OC323
Bizzarri Ranieri ... IL258
Bjorn Lars Olof ... P639
Blackburn Alison ... P624; P623
Blais Mylene ... OC308
Blankenburg Sandra ... IL148
Blaquière Sarah ... P607
Blázquez A. ... P719; P717; P705; P702
Blumthaler Mario ... IL317
Boateng Godfred ... OC243
Boeckmann Lars ... IL148
Bogdanska Barbara ... OC323
Bogomolni Roberto ... IL519
Boiy Annelies ... OC111
Bonetti Cosimo ... OC520
Bonneau Stéphanie ... P724; OC314
Bonsted Anette ... P736; IL241
Bontems Sébastien ... P704; IL139
Bornman Janet F. ... PL401
Borská Veronika ... P746
Boscá Francisco ... P637; OC336
Bouffler Simon ... P606
Bourg-Heckly Genevieve ... OC505
Bourre Ludovic ... P706; OC116; OC114
Bown Stephen G. ... OC245; PL201
Boyle Jennifer ... IL147
Boyle Ross William ... IL426
Brabec Viktor ... OC224
Branchini Bruce ... IL160
Brault Daniel ... P724; OC314
Bregante Monica ... IL232
Breitkreuz Helena ... P646
Brenn Thomas ... OC327
Bressenot Aude ... OC141

Brettel Klaus....OC107
Bretterklieber Agnes....OC329
Brewer Molly....IL503
Brie David....OC337
Bristow Carrie-Anne....IL426
Britton George....IL251
Brooke Rebecca.... OC328; OC115
Brown S.B.....IL428
Brownrigg Margaret M. P624; P623; OC328; OC327; OC125
Brubakk Alf O.....P734
Brühl Christoph....P622
Bruijnzeel Piet L.B.....P609
Bruns Thomas....IL128
Brunsvik Anders....OC338
Bruzell Ellen M.....P752; P630; P629; OC223
Bryant Lisa....IL440
Buchner Johannes....OC307
Bückle Rainer....IL131
Budzinskaya M.....P708
Buglass Suranahi K....OC356
Bujold Kenneth....P750
Buma Anita G. J.....IL360
Bunhill Lucy V....P621; OC320; OC207
Buriankova Luboslava....P737
Burne Allan....IL440
Busso Nathalie....OC422
Butchart Neal....P622
Buttani Valentina....P647
Buytaert Esther....IL138
Buzova Diana....P737
Byrdin Martin....OC107
Byrne Scott N.....OC221; IL120

C

Cadet Jean....P604; IL511; IL405; OC106; IL105
Caffieri Sergio....IL332
Calzavara-Pinton Piergiacomo....IL350; OC339
Cambrou Jacques....P703
Campbell Sandra....OC341
Campo Marino Andres....OC422
Cañete Magdalena....P731
Canguilhem Bruno....P607; P605
Cao Zhen....P647
Capobianco Massimo....OC334
Cardin Christine J.....P603
Carell Thomas....IL104
Carrilho Emanuel....P710
Carvalho C.M B.....P642
Casas Adriana....P726; OC113
Castano Ana P....IL418
Castell José Vicente....OC510
Cavaleiro J.A.S.....P719; P717; P642
Cavicchioli Rick....OC365
Cercignani G.....P616
Cevenini Luca....IL160
Chamorovsky Constantine....P657
Chamorovsky Sergei....P657
Chan Carling Y-Y.....OC126
Chang S.K.....IL137
Charvéron Marie....P607; P605; OC353
Checcucci Giovanni....IL258; IL235
Chee Soo Khee....P727
Chen Duan....OC338
Chen Wei R.....IL419
Cheung Lawrence H.....OC345
Chiaviello Angela....OC342
Chignell Colin F.....IL513
Chin William WL....P723
Chipperfield Martin....P622
Chobaz-Péclat Veronique....OC422
Chorvat Dusan jr.....OC313
Christensen Terje....P629; OC223
Church Martin....OC115
Chuzel Franck....P703
Claerhout Sofie....OC436
Clarke Kristy....P636
Climent María José....P633
Clough Geraldine....OC115
Colombetti Giuliano....P616; IL258
Colussi Valdir....OC142
Comelli Daniela....IL502
Comerci Diego J.....IL519
Connelly Sandra J....OC306
Conway Clare Louise....OC242
Cooper Kevin D.....OC142
Corrochano Luis M.....IL516
Cotterell Lindsey F....OC327
Coupienne Isabelle....P704
Crumrine David S.....P718
Cruz Nadya....P631
Cubeddu Rinaldo....IL502
Cuevas J.....P705
Čunderlíková Beata....IL110
Cunha M.A.....P642
Cuquerella M. Consuelo....OC336
Curnow Alison....OC346; OC341; OC140
Curylo Aleksander....OC323

D

D'Hallewin Marie Ange....OC112
Da Silva David....P728
Dąbrowski Janusz M.....P720
Dahl Jon E.....P630
Dameris Martin....P622
Damian Diona L.....OC219
Damiani Elisabetta....P627; OC354
Danesini Gianmaria....IL502
Dardalhon Delphine....IL404
Darvish Dana....IL108
Davies Michael J.....P614; IL512; OC407
Davies R. Jeremy H.....P603
Davis Scott C.....IL312
de Bruijn Henriette S.....IL504; IL109
de Graaff Marco....IL360
de Gruijl Frank R....IL431; IL118
De Gubernatis Enrico....IL235
de Haas Ellen R.M.....IL109
De Petrocellis Luciano....IL257
de Witte Peter A.M.....OC222; OC111
Declercq Lieve....P628
Dedic Roman....OC313
Deeni Yusuf Y....OC256
Defain María Victoria....OC113
Delie Florence....OC244
Den Outer Peter....OC321
Deniset Ariane....P745
Denk Helmut....OC307

Deo Shivashni S OC228
Dewaele Michael IL138
D'Hallewin Marie-Ange OC337
Di Giovanna John J. IL147
Di Mascio Paolo IL511
di Salvo L. P616
Di Venos G. P726
Di Venosa Gabriela OC113
Diaspro Alberto IL258
Diaz-Espinosa Yisaira P739
Diffey Brian IL324
Dimou Aikaterini P626; OC507
Dimou Elke OC416
Dini Fernando IL235
Dixon Katie M. OC228
Dizge Meltem Goksel P725
Dodeller Marc P728
Dogra Yuktee OC346
Dogru Murat P729
Dolp Frank IL130
Domanski Diane OC124
Domingo Diana Santo OC142
Douillard Samuel P749; P632; OC344
Douki Thierry P601; IL405; IL404; OC365; OC106; IL105
Dowdy John C. IL202
Dragland Inger Sofie P752; OC223
Dridi Walid OC308
Drouin Regen OC308
Durkin Marie T. P621; OC320; OC207
Durmus Mahmut P729; P725; P714
Durrant James IL151

E

Edwards Ana Maria P738
Edwards Gavin IL406
Eggleston Ian M. P706; OC116; OC114
Ehrenberg Benjamin IL311
Einem Björn v. IL130
Eker Andre P.M. OC107
El Batanouny M.H. P711
El Gohary E.S. P711
El Khatib Sami OC337
Elfeky Souad Ahmed P641
Elsner Peter IL131
Emmert Steffen IL148
Encinas Susana P633
Engelsen Ola IL203
Enk Claes D. P748
Eschwege Pascal P745
Esimbekova Elena N. OC161
Eslava Arturo P. IL516
Espagne Agathe OC107
Essen Lars-Oliver IL518
Ettler Karel OC322
Ewers Christine P651

F

Fabianova Hana P644
Fabre Michèle P745
Faccenda Filippo IL233
Fajardo Maureen P738
Falk Heinz OC222
Farr Peter M OC256
Faustino M.A.F. P719; P717; P642
Favre Gilles P607; P605
Fedorov F. P708
Feister Uwe OC321
Ferguson James P701; P602; OC256; OC224
Ferlicot Sophie P745
Fernandes S.C.D. P642
Ferrario Antonella OC343
Ferreira Juliana P740
Ferrero Louis OC357
Fetni Raouf OC308
Filho José Dirceu Vollet P740
Filipe Paulo Leal IL331
Finlay-Jones John J IL119
Flors Cristina IL310
Fontaine-Aupart Marie-Pierre P745
Forbes Paul Donald P703
Fort Sandrine Lévêque P745
Fotinos Nicolas OC337
Francesconi Stefania IL404
François Aurélie OC141
Frank H.A. IL246
Franke Leonora OC230
Friedmann Peter OC115
Fritsch Clemens P748
Frochot Céline P721; IL240
Fuentealba Denis P613
Fukuda Haydée P715; OC113

G

Gabriel Doris OC422
Gaertner Wolfgang P647
Galaris Dimitrios P626; OC507
Gambale Franco IL232
Gan Yu P603
Gandolfi Andrea IL233
Garaczi Edina IL413
Garcez Aguinaldo S IL424
Garcìa Angélica P738
García Carmelo P631; IL333
Garcia-Ramos Jose Vicente P735
Gariba Munir Antônio P615
Garmyn Marjan P628; OC436; IL252
Garnock-Jones Phil IL440
Garoli D. OC659; OC658; OC358
Garrier Julie OC141
Gasparutto Didier P604; IL511
Gauslaa Yngvar OC442
Gavalda Salvador D P638
Gbur Peter OC313
Gederaas Odrun A. P734; OC338
Ghetti Francesco IL235
Gibbs Neil K. P640; P636; P625; P624; P623; OC328
Gibbs Summer L. IL312
Giorgetta M. A. P622
Girard Pierre-Marie IL404
Girotti Albert W IL514
Giuntini Francesca P706; OC116; OC114
Glaeser Jens OC515; OC367
Glaeser Stefanie OC367
Glanzmann Thomas OC508
Gledhill K. OC125
Goldbaum Fernando A. IL519
Gollnick Sandra O. IL421
Gomer Charles J. OC210; IL136

Gomes A.T.P.C. P642
Gómez-Lechón Maria José........ OC510
González S. P705
Goralczyk Regina........ IL250
Gorman Shelley........ OC220; IL119
Gracanin Michelle........ P614
Gravemann Ute P612
Greci Lucedio........ P627; OC354
Greinert Ruediger........ OC437
Greinix Hildegard........ OC416
Griffiths Christopher E.M. P625; OC327
Griffiths J. IL428
Grifoni Daniele........ IL319
Groenen E.J.J. IL246
Grondelle Rienk van........ OC520
Groot Marie-Louise........ OC520
Grossart Hans-Peter........ OC367
Groth Norbert........ OC357
Gudgin-Dickson Eva P722
Guedenet Jean-Claude........ OC337
Gueranger Quentin IL305
Guillemin François.... P728; P721; OC337; IL240; OC141; OC112
Gurek Ayse Gul........ P729; P725; P714
Gurny Robert........ OC422; OC417; OC244
Gustavsson T. IL102

H

Haar Manuela OC329
Häder Donat-Peter........ P649; IL359; IL234
Haenssle Holger IL148
Hains Peter G. OC407
Hala Jan........ OC313
Halliday Gary M.OC228; OC221; OC219; OC150; OC126
Halsbeck Martin OC307
Hamblin Michael R IL424; OC423; IL418
Hammond Kirsten J.L. OC126
Harbottle Andrew........ OC226
Hart Prue H OC220; IL119
Hasan Tayyaba P709; OC429; OC347; IL238; IL137
Haukvik Tone........ P752
Haure Marie-José OC353
Hawk John L M........ IL408; IL326; IL145
Haylett A. OC125
He Yu-Ying........ IL513
Heberle Joachim........ P646; OC521
Heczko Piotr Bogumł,........ P707
Hegemann Peter OC520
Heitman Joseph IL516
Helbling E. Walter........ P650; IL360
Heng Paul WS P723
Heringova Pavla OC224
Hermida Laura........ OC113
Hernandez-Pigeon Hélène........ P607; OC353
Hideg Éva........ IL253
Hill Claire........ P701
Hjelde Astrid P734; OC338
Hoberman Alan M. P703
Hofer Angelika........ OC415; OC329
Hoffmann-Wülfing Karena IL439
Hofkens Johan........ IL310
Høgset Anders........ IL241
Hönigsmann Herbert IL409
Hsia Andrew........ OC142
Hu Ping OC243
Huang Xiao Xuan OC150
Huber K. OC328
Hunter Christopher Neil........ IL152
Huntosova Veronika P737; P733

I

Ibbotson Sally H OC256
Ibrahium M.K. P711
Idnurm Alex........ IL516
Iga Arthur........ P743
Iglesias-de la Cruz M.C. P705
Ilik Petr P652
Imasato Hidetake P710
Immeln Dominik........ OC521
Imoto Kyoko IL147
Inamori Yoshihiro P634
Ishimura Kazunori P716
Ivancic Erika OC416

J

Jaen P. P705
Jamie Joanne F. OC407
Jancura Daniel........ P737; P735; OC313
Jańczyk Agnieszka........ P707
Janig Elke........ OC307
Janknegt Paul P. IL360
Janouch Michal OC322
Janssens A Soe........ IL118
Jarošová Jana P746
Javeri Arash OC150
Jenkins Gareth........ IL363
Jeong Daewoong........ P656
Jichlinski Patrice OC506
Jiménez-Banzo Ana IL310
Johansson Ann OC134
Johansson Thomas OC134
John Susan OC123
Johnsen Bjørn P629
Johnston Brian P602
Jones Christophe........ P608; IL330; IL303
Jørgensen Henrik L. OC208
Jørgensen Niklas Rye........ OC208
Jori Giulio P738; IL425; IL233
Joux Fabien P601; OC365; IL105
Juarranz A. P726; P719; P717; P705; P702
Judge Melinda........ OC220
Julkunen-Tiitto Riitta OC364
Just Ulrike........ OC416
Juzeniene Asta OC340; OC315

K

Kaatz Martin IL131
Kaina Bernd IL302
Kandela Siham A OC430
Kaneko Kazuyo........ OC123
Kaneko Sadao IL211
Kang Joonho P656
Karakhan V.B. OC212
Karatani Hajime........ OC162
Kasahara Shigeru P634
Kaskakova Slavka........ IL504; OC218
Kasparkova Jana OC224
Kawashima Norimichi P741

Kaxiras Efthimios....................OC507
Kaykova Elisaveta V....................P645
Kazantzidis Andreas....................P622; OC321
Kemeny Lajos....................IL413
Kennis John T.M....................OC520
Kerl Helmut....................OC415; OC329
Khan Sikandar G....................IL147
Kift Richard....................P621; OC320; OC207
Kim Daeshik....................P656
Kim Sang-in....................IL403
Kimlin Michael G....................P617
Kirkevold Håvard....................P736
Kisch Horst....................P707
Klar Tobias....................IL518
Klein Frank....................OC357
Klementova Sarka....................P643; OC237
Klisch Manfred....................P649
Klosner Gabriele....................OC416
Klug Gabriele....................OC515
Knobler Robert....................OC416; IL411
Knox Peter....................P657
Kochevar Irene....................P750; IL255
Kockott Dieter....................OC357; OC230; OC132
Koepke Peter....................P619; IL318
König Karsten....................IL131
Kopacek Jiri....................OC237
Kopera Daisy....................OC415
Korbelik Mladen....................IL420
Koreck Andrea....................IL413
Korytowski Witek....................IL514
Koskela Tapani....................OC321
Kosobe Toshiyuki....................P741
Kostron Herwig....................IL215
Kotilainen Titta Katariina....................OC364
Kotova Elena A....................P712
Kottke Tilman....................P646; OC521
Kovacs G....................P611
Kowalczuk Christine....................P606
Kozhinova Elena A....................P747; OC225
Kozir Lyudmila A....................P747
Kozmin Stanislav....................IL404
Kraemer Kenneth H....................IL147
Krammer Barbara....................IL209
Kratasyuk Valentina A....................OC161
Krishnan Rajagopal....................OC227
Kriska Tamas....................IL514
Kriz Dalibor....................OC237
Krokan Hans E....................P734; OC338
Krueger Ullrich....................IL148
Kruijt Bastiaan....................IL504; OC218
Krutmann Jean....................IL149
Krzyscin Janusz....................OC323
Kudryasheva Nadya S....................OC163
Kukielczak Barbara M....................IL513
Kuritzky Alexandra....................OC220
Kuschal Christiane....................IL148
Kuvshinov Y.P....................OC212
Kuzmin S....................P708
Kuznecov V.V....................OC212
Kyagova Alla A....................P747; OC225

L

Labarussiat Anita....................IL330
Lachireddy Kishen....................OC124
Lackner Bernd....................OC222
Lademann Jürgen....................OC357
Lagunas Marcos....................P650
Lajos Gejza....................P735
Lang Jaroslav....................P652
Lange Norbert....................OC422; OC417; OC244; IL239
Langematz U....................P622
Lapeta Bozena....................OC323
Larsen Eivind La Puebla....................OC338
Larsson Per....................OC442
Laspe Petra....................IL148
Lassalle Henri Pierre....................OC112
Latulippe Katie....................P722
Lau Weber KO....................P723
Lazzarotto E....................IL102
Le Harzic Ronan....................IL131
Learn Douglas Brian....................P703
Lécart Sandrine....................P745
Lee John....................IL158
Lee Michael P. H....................P603
Lee Peter L....................P609
Legat Franz J....................OC415; OC329
Lerche Catharina M....................IL434
Lhiaubet-Vallet Virginie....................PL101
Liang A....................IL137
Lim Pei Li....................P723
Limon-Flores Alberto Y....................IL120
Lin Z....................P642
Ling Tsui C....................OC328
Litynska Zenobia....................OC323
Lobanov Andrej V....................P730
Loschenov V....................P708
Losi Aba....................P647; IL517
Lottspeich Friedrich....................OC515
Lovisa Blaise....................OC506
Lucia Sabina....................P616; IL258
Lugtenburg J....................IL246

M

Macdonald Linda J....................OC221
Mackay Fiona S....................OC224
MacRobert Alexander J....................P743; P732; P706; OC245; OC116; OC114; OC113
Macyk Wojciech....................P707
Maekawa Michiko....................P634
Maes Daniel....................P628
Magaraggia Michela....................IL233
Mai Z....................IL137
Mailhos Carolina....................OC417
Majora Marc....................IL149
Malakhov Mikhail V....................P713
Maldant Basile....................P745
Malik Zvi....................IL108
Malley Roslyn C....................OC122
Malone John F....................P603
Mamikonyan V....................P708
Mancini Eva....................P622
Manet Ilse....................OC334
Manganoni Ausilia....................IL350
Manoli Francesco....................OC334
Mansurova Galina V....................P747; P713
Manzini E....................P622
Marchal Sophie....................OC141
Marcoval M. Alejandra....................IL360

Maresch Tanja IL149
Marguet S. IL102
Maria Roberta M. P710
Marin Maria Luisa OC510
Markovitsi D. IL102
Marrot Laurent P608; IL330; IL303
Martinet Wim IL138
Martinez Glaucia R. IL511
Mason Rebecca S. OC228; OC150
Masoodi M. OC125
Matallana Surget Sabine P601; OC365; IL105
Mateasik Anton P733
Mathes Tilo OC520
Matroule Jean-Yves IL139; IL138
Matsui Mary OC123
Matsumoto Shoji OC162
Maunit Benoit P728
McGarvey David J. P636
McGee Heather M OC122
McGlade Jacqueline P IL119
McKinley Alex Russell P617
McLeod Andrew OC441
McLoughlin Emma OC441
McNulty Liam OC320
Meador Jarah A. P601; OC365; IL105
Medeiros Marisa H.G. IL511
Meffert Hans OC414
Melconian Alic K OC430
Mellish K. IL428
Menck Carlos FM IL304
Mendía Luis P650
Menezes Priscila Fernanda Campos de P744; P740
Messina Nicola IL235
Meunier Jean-Roch P608; IL330; IL303
Mezzanotte Laura IL160
Michelini Elisa IL160
Mikes Jaromir P733
Miolo Giorgia IL332
Miranda Miguel Ángel P637; P633; OC510; OC336
Miskovsky Pavol P737; P735; P733; OC313
Mitchell David L IL413; OC306
Mitoraj Dariusz P707
Mizdrak Jasminka OC407
Moan Johan P751; OC340; OC315; OC229; OC206
Moeller Robert E. P648
Mohr Harald P612
Mojzisova Halina P724; OC314
Moldt Julia IL518
Moncrieff John OC441
Monteiro Carlos J.P. P720
Monti Marcello P742; OC343
Monti Sandra OC334
Moore Jason M. IL438
Moore Michael R. P617
Moreno-Swirc Sophie OC505
Morera Isabel M. P637
Morgan Janet OC348
Morisbak Else P752; OC223
Moriyama Lilian Tan P740
Morley S.M. IL428
Morlière Patrice IL331
Moseley Harry P701; P602; IL428; OC224
Moshnina Zoya I. P747
Motta Stefania P742; OC343
Mouret Stephane IL405; IL105
Mroz Pawel A OC423; IL418
Mullenders Leon H.F. PL501; IL431
Muller H Konrad OC122
Muller Jean François P728
Müller Thomas H. P612
Muñoz Marcelo P738
Murphy Gillian M IL325
Musio Carlo IL257

N

Nadova Zuzana P733
Naga M.I. P711
Nakamura Masao P634
Nakamura Michihiro P716
Nakamura Shingo P634
Navaratnam Suppiah OC335
Neale Patrick J. P648; IL362
Negrimovsky V. P708
Neidle Stephen P603
Nesland Jahn M. IL110
Neto A. J. Silva IL157
Neumann H A Martino IL109
Neves M.G.P.M.S. P719; P717; P642
Newsham Kevin OC441
Nicolaou A. OC125
Nicolosi P. OC659; OC658; OC358
Nield Jon OC153
Nielsen Kristian P. OC315
Nieves Ileana P631
Nijhof Joanne GW IL431
Nishijima Ken-ichi P635
Nohama Percy P615
Nonell Santi IL310
Nordlund Thomas M. OC227
Norman Anthony W OC228
Norum Ole-Jacob IL241
Norval Mary OC123
Nürnberg Bernd IL204
Nys Kris IL252

O

Ogilby Peter R. IL309
Ogilvie William P701
O'Grady C. IL428
Oh Kyu Seon IL147
O'Hara Julie A. IL312
Ohkura Kazue P635
Ohmiya Yoshihiro IL159; IL157
Ohta Shogo OC162
Okabe Toshihiro P634
Oleinick Nancy L. OC142
Olivier David P749; P632; OC344
Olivo Malini P727; P723
Olsen Kenneth W. P718; OC243
Onidas D. IL102
Orlando Pierangelo IL257
Ortel Bernhard IL349
Osaki Takashi OC162
Oseroff Allan R OC348
Oyola Rolando P631; IL333

P

Paget Timothy IL426

Pålsson Sara OC134
Palumbo Giuseppe OC342
Pandey Ravindra K. OC348
Paradis Isabelle OC308
Paris Cecilia P633
Paris Gastón IL519
Parisi Alfio V P618
Park Sanghyun P656
Parrinello Giovanni OC339
Pashkovskaya Alina A. P712
Paterno Fabiola IL233
Patrice Thierry P749; P632; OC344
Pattison David I. P614
Paul Nigel D. IL438; OC366; IL363
Pavel Stan IL118
Pawlak Anna IL249
Pelizzo M.G. OC659; OC658; OC358
Peng Qian IL241; IL110
Pereira Mariette M. P720
Pereira Steve IL214
Perez Philippe IL330
Perussi Janice R. P710
Pesce Mattia IL258
Peserico A. OC659; OC358
Pfeifer Gerd P. IL403
Phan Tai A. OC219
Philippe Perez P608
Philipsen Peter A. IL434; OC208
Phillips J.B. P732
Phillips-Kress Jesse P648
Piazena Helmut P610; OC414; OC230; OC132
Piette Jacques G P704; IL139; IL138
Pifferi Antonio IL502
Piñero Luis E. P631; IL333
Pitari Gianni P622
Plavsky V. P751
Plénat François P721
Poddybny B.K. OC212
Pogoda de la Vega Ulrike OC106
Pogue Brian W. IL312
Pokorny Richard IL518
Ponomarev Gelii V. P747; P713
Porcal Petr OC237
Porojnicu Alina Carmen OC229; OC206
Porter Jason P648
Pospisil Pavel P652
Potapenko Alexander Ya. P747; P713; OC225
Pottier Roy P722
Poulsen Thomas IL434
Pradines Anne P607; P605
Prata A.C.B. P642
Priestner Marisa P606
Proby Charlotte OC436
Puzhuoma Xiao OC340
Pye Andrew OC341; OC140

Q

Qiu Xuepeng P741
Quehenberger Franz OC329

R

Rainho J.P. P642
Ramoino Paola IL258
Rana Sabita OC221
Randeberg Lise Lyngsnes OC338
Raval Chintan IL406
Ravanat Jean-Luc P604; IL511; IL405
Redmond Robert P750
Reeve Vivienne E OC228; PL135; OC124
Regulus Peggy P604
Reichrath Jörg IL204
Reid Alexis Lynn P722
Reilly Siobhan M. P625
Reitz Günther OC106
Rello-Varona Santiago P731
Remedi A. P616
Renwick Yasmin J OC219
Reshetnikov Andrei V P713
Rettberg Petra OC106
Reynaud Claude-Agnès IL305
Reynaud-Angelin Anne IL404
Rhodes Lesley Elisabeth P624; P623; P621; IL428; OC355; OC328; OC327; OC320; OC226; OC207; OC125; OC115
Richards H.L. OC328
Riemann Iris IL131
Rivarola Viviana P715; P702
Rizvi I. IL137
Rizwan Muneeza IL428; OC355; OC226
Robe Pierre A. P704
Robert Bruno IL247; OC155
Roberts Joan Elizabeth OC121
Robinson Dominic J IL504; OC218; IL109
Robinson Gregory OC417
Rocha J. P642
Roda Aldo IL160
Rodríguez F. Sanz P705
Rodriguez-Blanco Isabel OC355; OC226
Rodriguez-Romero Julio IL516
Roelandts Rik IL352; OC111
Roelants Mieke OC222
Rohacova Jana OC510
Ronto G. P611
Rosenblum Michael G. OC345
Rotomskis Ricardas OC133
Rozanov E. P622
Rozanowska Malgorzata Barbara IL249
Rozanowski Bartosz IL249
Rueck Angelika IL130
Rumie B. P702
Rutter Kirsty J OC327
Ruzicka Thomas P748
Ryan Ken G IL440

S

Sadler Peter J OC224
Sage Evelyne IL404; IL105
Sala Raffaella OC339
Salama M.S. P711
Sallum Ulysses Wilson P709; OC429; IL238
Salomon Yoram OC216
Sambuco Christopher Paul P703
Sanchez-Cortes Santiago P735
Sanjeevaiah Prakash P718
Santillo Silvia IL257
Santus René IL331
Sanz Catalina IL516
Sanz-Rodríguez F. P726; P719; P717; P702

Sarasin Alain IL305
Saunders Rick P606
Saydan Nil P729; P725; P714
Sayre Robert M. IL202
Schenke-Layland Katja IL131
Scherz Avigdor OC216
Schlesinger Ramona OC521
Schmalwieser Alois W. P619; IL316
Schneckenburger Herbert IL128
Schouten Peter W P620
Schröder Peter IL149
Schultze Matthias P653; IL439
Seifalian Alexander M. P743
Seki Koh-ichi P635; P634
Selbo Pål Kristian OC345; IL241; OC143
Senhorinho Halina Camargo P615
Sepic Goran OC329
Seppelt Rod IL440
Serra V. Vaz P719; P717
Sherratt Michael J. P625
Shevchik S. P708
Shima David T. OC417
Shishido Naomi P634
Shono Masayuki P716
Sidhu Meneka OC115
Sidoroff Alexis IL213
Sik Robert H. IL513
Silva Eduardo P613
Sippel Rüdiger OC132
Skórska Elżbieta P655; P654
Slaper Harry OC321; OC146
Smaers Katrien P628
Smetana-Just Ulli OC123
Smirnova Violetta IL404
Smith Gillian OC256
So Alexander OC422
Sobrino Cristina P648; IL362
Soh Kwangsup P656
Sokolenko Elena A. P712
Sokolov Valeri S. P712
Solban N. IL137
Solhaug Knut-Asbjorn OC442
Song Pill-Soon PL301
Southworth Tara IL160
Spinelli Lorenzo IL502
Stark Katharina OC230
Stary Anne IL305
Steenkeste Karine P745
Steindal A.H. P751
Steiner Rudolf IL130
Steinmetz Cornelia IL130
Stepinac Thomas OC347; IL137
Sterenborg Henricus J.C.M. IL504; OC218; IL109
Steuer Heiko IL128
Stochel Grażyna P720; P707
Stockert Juan Carlos P731; P726
Stracke Frank IL131
Strakhovskaya M. P708
Strange Richard C IL205
Strauss Wolfgang S.L. IL128
Strus Magdalena P707
Sugiyama Hiroshi IL103
Suhling Klaus IL129
Sukovataya Irina E. P645
Sülflow David OC230
Svaasand Lars Ottar OC338
Svanberg Katarina OC134
Svanberg Sune OC134
Swartling Johannes OC134
Swartz Trevor E. IL519
Szwarc Wiktor P655

T

Tagua Victor G. IL516
Takahashi Masayuki P635
Takei Yasushi P741
Talbot F. IL102
Tamura Deborah IL147
Tanew Adrian IL410
Taroni Paola IL502
Taskova Rilka IL440
Tegelberg Riitta OC364
Tegos George P IL424
Telfer Alison IL156
Tessier Ludovic IL404
Thiberville Luc OC505
Thieden Elisabeth OC208
Thody A.J. OC125
Thomas Noémie P721; IL240
Thoms Kai-Martin IL148
Tikhomirov Andrei M. OC225
Tilgen Wolfgang IL204
Tirand Loraine P721; IL240
Tobin D.J. OC125
Tokuoka Yoshikazu P741
Tomé A.C. P719; P717; P642
Tomé J.P.C. P719; P717; P642
Tønnesen Hanne Hjorth P752; OC223
Torricelli Alessandro IL502
Tourpali Kleareti P622
Trautinger Franz OC416
Traynor Nicola J P701; P602
Truscott George P636; IL248
Truscott Roger J.W. OC407
Tuncer Sinem P729; P714
Turnbull David John P620; P618
Tye Joanne P624
Tyrrell Rex Michael IL406

U

Uchugonova Aisada IL131
Uebelhack Ralf OC230
Ueda Takahiro IL147
Ugalde Rodolfo A. IL519
Ullrich Stephen E. IL433; IL120
Ungiadze G.V. OC212
Urbańska Krystyna P720
Usai Cesare IL258
Utzinger Urs IL503
Uzdensky Anatoly B. P730; OC144

V

Vakulovskaya E.G. OC212
van den Bergh Hubert OC506
van der Ploeg-van den Heuvel Angelique OC218
van Dijk Arjan OC146
van Hemert M.C. IL246
Van Kelst Sofie IL138

Van Laethem An OC436; IL252
van Stokkum Ivo H.M. OC520
van Weelden Huib P609
van Wijnen Harm OC146
Vanderesse Régis P721; IL240
Vasovič Vlada IL110
Vecerova Kristyna P652; OC442
Venäläinen Tuulia OC364
Venditti Elisabetta P627
Venius Jonas OC133
Venturini Marina IL350; OC339
Verdebout Jean IL318
Verma Ajit K IL432
Verma Sarika P709; OC429; IL238
Verschooten Lien P628; OC436
Vever-Bizet Christine P724; OC505; OC314
Vicidomini Giuseppe IL258
Villafañe Virginia E. IL360
Villanueva Angeles P731
Villette Sandrine OC107
Visser Ronald J. W. IL360
Viviani Vadim R. IL157
Volanti Cédric IL139
Volkmer Beate OC437
Vorobey A. P751
Vorobey P. P751
Vorozhtsov G. P708
Vrablikova Hana OC442
Vysotski Eugene S. IL158

W

Wackernagel Alexandra OC329
Wagner Michael IL128
Wagnières Georges A. OC508; OC506
Wahlqvist Rolf IL110
Walawender Jakub OC323
Walker Susan Lesley OC123; IL117
Walt Heinrich OC217
Wang Tzu-wen OC245
Wargent Jason J. IL438; OC366; IL363
Warloe Trond IL110
Waser Mario OC222
Watson Rachel E.B. P625; OC355; OC327; OC226; OC115
Watt Gregory OC429
Webb Ann R. P621; OC320; OC207
Weber Petra IL128
Weill Jean-Claude IL305
Weiss Aryeh IL108
Wencierska Maja P654
Werner Sabine IL254
Weyergang Anette IL241; OC143
Wilkie Murray JV OC256
Williamson Craig E. IL361; OC306
Wilson Michael P706; IL427; OC116; OC114
Winhoven Sandra M. P640; P624; P623
Winyard Paul OC346
Wirtz A.C. IL246
Wojtyk James T.C. P722
Woldt M. OC321
Wolf C Roland OC256
Wolf Eva P646
Wolf Peter OC415; IL412; OC329; OC307
Woods Gregory M OC122
Woods Julie A. P701; P602; OC224
Wright K.E. P732
Wu Chun IL159
Wulf Hans Christian IL434; OC208

Y

Yaghini Elnaz P743
Yamamoto Tadamiti P634
Yao Min P750
Yasui Masashi OC162
Young Antony R OC356; OC123
Yslas I. P702
Yuen Gan Yap-Yik P727

Z

Zachmann Karolin IL148
Zaitoun Basel P718
Zamarrón A. P719; P717; P702
Zane Cristina OC339
Zastrow Leonhard OC357
Zatloukal Kurt OC307
Zeisser-Labouèbe Magali OC244
Zemanova Martina P643
Zhang Wendy OC345
Zhao Chun-Mei OC338
Zheng Xiang IL238
Zhong Julia IL406
Zhu Chengming OC435
Zipoli Gaetano IL319
Zobawa Monica OC515
Zonios George P626; OC507
Zuluaga Maria Fernanda OC417