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Dear Colleagues and Friends,

It is my pleasure to welcome you to the 10th Congress of the European Society for Photobiology in Vienna, Austria.

As with previous meetings, the programme of the 10th Congress covers all major fields of Photobiology. There will be a balanced mixture of photobiology updates, special lectures, symposia, workshops, and poster sessions. We are particularly pleased to be able to include in our programme for the first time a joint symposium co-organised by the European Photochemistry Association (EPA) and the ESP. This reflects the close association that exists between the two societies, which, besides many shared research interests, have in common the joint ownership of the new official journal, *Photochemical and Photobiological Sciences*. The Organising Committee has tried to make up a programme of topics, with both local and international chairpersons and we have received over 400 contributed papers for this meeting, which is open to all colleagues interested in photobiology.

The Local Organising Committee has put together a social and cultural programme for delegates and their accompanying guests. For an extension of your stay in Austria we should like to recommend the optional excursions that are offered by our travel agency.

Finally, I would like to thank our sponsors. Full recognition of their contribution will be made at the meeting and in the Congress Programme Booklet.

Thank you for coming to Vienna to celebrate with us the 10th Congress of our Society.

Herbert Hönigsmann, MD
Local Chairman
**Congress Venue**
Hörsaalzentrum, Vienna General Hospital
AKH- ("Allgemeines Krankenhaus der Stadt Wien")
Währinger Gürtel 18-20
A-1090 Vienna

**Congress Offices**
Scientific and Administrative Secretariat
Vienna Medical Academy
Mrs. Birgit Kruse
Alser Strasse 4
A-1090 Vienna, Austria
Phone: +43-1-405 13 83 11
Fax: +43-1-407 82 74
e-mail: office@esp2003.org

**Travel and Hotel**
Accommodation
Mondial Congress
Faulmannsgasse 4
A-1040 Vienna, Austria
Phone: +43-1-588 04-0
Fax: +43-1-5869185
e-mail: novak@mondial.at

**Exhibition Management**
Medizinische Ausstellungs- und Werbegesellschaft
Freyung 6/3
A-1010 Vienna, Austria
Phone: +43-1-536 63-0
Fax: +43-1-535 60 16
e-mail: maw@media.co.at

**Registration**
Opening hours registration desk
Saturday, September 6th, 2003 16.00 – 19.00
Sunday, September 7th, 2003 08.00 – 18.00
Monday, September 8th, 2003 08.30 – 19.00
Tuesday, September 9th, 2003 08.30 – 19.30
Wednesday, September 10th, 2003 08.30 – 19.00
Thursday, September 11th, 2003 08.30 – 14.00

**Registration Fees**

<table>
<thead>
<tr>
<th>Payment received</th>
<th>before June 1, 03</th>
<th>before August 15, 03</th>
<th>On-site Payment</th>
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<tr>
<td>ESP-Members</td>
<td>Euro 300.–</td>
<td>Euro 400.–</td>
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<td>ESP-Members (Eastern Countries)</td>
<td>Euro 225.–</td>
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<td>Euro 180.–</td>
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* Students: Letter from Supervisor required.
Registration Fee Includes:
Scientific Sessions, Exhibition, Programme and Abstracts, Coffee Breaks, Welcome Reception and Dinner at a Viennese “Heurigen”.

Accompanying Fee Includes:
Welcome Reception and Dinner at a Viennese “Heurigen”

Cancellation Policy
Cancellations are to be made in writing or by fax to Vienna Medical Academy. Payment regarding registration fee, and accompanying persons’ programme will be refunded as follows.

Cancellation received
before August 11th, 2003 75% refund
after August 11th, 2003 no refund

All refunds will be processed after the Congress.

ESP-Membership fees for 2003 in EUR
“Photochemical & Photobiological Sciences” (PPS) is the official journal of ESP published by the Royal Society of Chemistry (RSC), UK, and is available both in electronic and hardcopy versions

<table>
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<td>4 years</td>
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Electronic version | Electronic plus hardcopy | Membership only |
--------------------|--------------------------|-----------------|
40.– EURO 1 year   | 95.– EURO 1 year         | 15.– EURO 1 year |
70.– EURO 2 years   | 180.– EURO 2 years       | 25.– EURO 2 years |
135.– EURO 4 years  | 355.– EURO 4 years       | 45.– EURO 4 years |

Official Language
The official congress language is English. There will be no simultaneous translation.

CME Credits
The Austrian Medical Association has certified the “10th Congress of the European Society of Photobiology” for the CME programme with 30 credits.

Social Programme
Welcome Reception at the Foyer of the General Hospital Vienna, “Hörsaalzentrum” Währinger Gürtel 18–20, 1090 Vienna. Saturday, September 6th, 2003 at 18.00 hrs.

Dinner at the Viennese “Heurigen” Fuhrgassl-Huber, Neustift/Walde 68, 1190 Vienna. Tuesday, September 9th at 19.00 hrs. Registration required

Upon the invitation of the mayor of the city of Vienna.

Bus transfer: There will be a bus transfer to the “Heurigen”. Meetingpoint: 18.45 hrs. Departure at the main entrance of the General Hospital. Return transfer will be provided.

Young Scientist Award
The Young Scientist Award of the European Society of Photobiology is sponsored by the Royal Society of Chemistry and the ESP.
Medal Awards

ESP Medals will be awarded to:

Jan C. van der Leun for long, dedicated and outstanding scientific contribution within the field of photobiology.
Rex M. Tyrrell for outstanding scientific contribution within the field of photobiology.
Christoph Abels, Young Scientist Award, for outstanding research and contribution to photobiology.

Lunch

Lunch vouchers are available at the registration desk. Lunch will be served in the lobby of the congress venue. Please bring your lunch voucher to the service point.
Price for one voucher: € 7.– (one drink included)

Lottery

There will be a lottery for the “Handbook of Organic Photochemistry and Photobiology” Publisher: CRC Press, 2000 N.W. Corporate Blvd., Boca Raton, FL 33431 USA. You have the possibility to try your luck at the registration desk!

Information for Authors

Invited Lectures (IL), Free Communications (FC) Sessions and Poster Sessions

Presentation time for Invited Lectures: 30 minutes (incl. discussion),
Presentation time for Free Communication Presentations: 15 minutes (incl. discussion).

All lecture rooms are equipped with double slide and PowerPoint projection facilities.
Poster viewing will be on Monday, 8th September and on Wednesday, 10th September between 14.15 – 15.30 in the Poster Exhibition Area.
Authors are requested to stay at their poster within this time to present their work.

Slide Preview

Slide and power-point preview is possible in Room 12.
Slides and power-point files must be handed over in the Preview Room at least one hour before the session start.
### ESP 2003 Sponsors

<table>
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### ESP 2003 List of Exhibitors (as per printing date)

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<tr>
<td>Ärztezentrale, Adressen- u. Drucksortenverlag, Austria</td>
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<td>La Roche Posay, Austria</td>
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<td>Vichy Laboratories, Austria</td>
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<td>Waldmann Medizintechnik, Waldmann Medical Division, Germany</td>
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International Scientific Committee

Patricia Agostinis, BE  
Stefan Andersson-Engels, SE  
Eva-Mari Aro, FI  
James Barber, UK  
Reinhold Baumgartner, DE  
Homer Black, USA  
Giovanni Bottiroli, IT  
Michael Boulton, UK  
Silvia Braslavsky, DE  
Stanley Brown, UK  
Jean Cadet, FR  
Piergiacomo Calzavara-Pinton, IT  
Giovanni Checucci, IT  
Rosalie Crouch, USA  
Francesco Dall’Acqua, IT  
Frank R. de Gruijl, NL  
Guido De Guidi, IT  
Chris Edwards, UK  
Benjamin Ehrenberg, IL  
James Ferguson, UK  
Wolfgang Gärtner, DE  
Paolo Giacomoni, USA  
Neil Gibbs, UK  
Tomas Gillbro, SE  
Norbert Hampp, DE  
John Hawk, UK  
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Éva Hideg, HU  
Erhard Hölzle, DE  
David Hunt, UK  
Gareth Jenkins, UK  
Giulio Jori, IT  
Nick Kollias, USA  
Mladen Korbelik, CA  
Michael Landthaler, DE  
Francesco Lenci, IT  
Richard L. McKenzie, NZ  
Ana Moore, USA  
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Mary Norval, UK  
Bernhard Ortel, USA  
Allan Oseroff, USA  
Giuseppe Palumbo, IT  
Thierry Patrice, FR  
Elena Reddi, IT  
Petra Rettberg, DE

Lesley Rhodes, UK  
Andre Rougier, FR  
Evelyne Sage, FR  
Alain Sarasin, FR  
Tadeusz Sarna, PL  
Herbert Schneckenburger, DE  
Thomas Schwarz, DE  
Gunther Seckmeyer, DE  
John Simon, USA  
Ray Smith, USA  
Per Söderberg, SE  
Uwe Sonnewald, DE  
Herbert Stepp, DE  
Lars Svaasand, NO  
Rolf-Markus Szeimies, DE  
Paola Taroni, IT  
T. George Truscott, UK  
Rex M. Tyrrell, UK  
Beate Uhlmann, DE  
Dennis Valenzeno, USA  
Maria Vernet, USA  
Mark Wainwright, UK  
Antony Young, UK

Organizing Committee

Janet F. Bornman (DK), Chair and President of the ESP  
Kristian Berg (NO), Officer of the ESP  
Francesco Ghetti (IT), Treasurer of the ESP  
Jacques Piette (BE), President Elect of the ESP  
Herbert Höögismann (AT), Local Chairman  
Robert Knobler (AT), Local Organiser  
Franz Trautinger (AT), Local Organiser

Business Meetings

Executive Committee I: Saturday, September 6th, 2003  
(Meeting point: registration desk)  
12.00 hrs.

IUPB Board Meeting: Sunday, September 7th, 2003  
(Meeting point: registration desk)  
17.30 hrs.

ESP Editorial Board Meeting: Monday, September 8th, 2003  
19.00 hrs. Room 4

General Assembly: Tuesday, September 9th, 2003  
17.15 hrs. Room 1

Executive Committee II: Wednesday, September 10th, 2003  
(Meeting point: registration desk)  
19.00 hrs.
Saturday, 6th September 2003

16.00
Registration

18.00
Welcome Reception

Sunday, 7th September 2003

Room 1 09.00 – 09.45
OPENING CEREMONY
WELCOMING ADDRESS
Janet F. Bornman, ESP President; Herbert Hönigsmann, Chair of Local Organising Committee
10th ESP CONGRESS – HISTORICAL ASPECTS
Kurt Schaffner, Founding Member of the European Society for Photobiology:
PRESENTATION OF “PHOTOCHEMICAL AND PHOTOBIOLOGICAL SCIENCES”
Giulio Jori, Jamie Humphrey and Jacques Piette

09.45 – 10.15
YOUNG SCIENTIST AWARD LECTURE
IL001 TARGETING OF THE VASCULAR SYSTEM OF SOLID TUMORS BY PHOTODYNAMIC THERAPY
C. Abels (Heidelberg, Germany) introduced by J. F. Bornman

10.15 – 10.30 Coffee Break

Room 4 10.30 – 13.15
PHOTOSYNTHESIS
Chairs: A. Moore (Tempe, United States); J. Barber (London, United Kingdom)

IL002 MIMICKING THE PHOTOSYNTHETIC FUCTIONS OF CAROTENOID POLYENES
A. L. Moore1, G. Kodis1, P. A. Liddell1, R. Palacios1, W. Thompson1, S. Gould1, J. T. M. Kennis2, R. van Grondelle2, A. N. Macpherson3, T. Gillbro3, T. A. Moore3, D. Gust1
(1Tempe, United States, 2Amsterdam, The Netherlands, 3Umeå, Sweden)

IL003 HOW PLANTS SEE BLUE LIGHT. INSIGHTS FROM TIME-RESOLVED SPECTROSCOPY
J. T. M. Kennis (Amsterdam, The Netherlands)

IL004 FUNCTIONS OF CAROTENOIDS AND OTHER PIGMENTS IN PHOTOSYSTEM II FROM A STRUCTURAL POINT OF VIEW
J. R. Shen2, N. Kamiya2 (1Okayama, Japan; 2Hyogo, Japan)

IL005 SIDE-PATH ELECTRON DONORS IN PHOTOSYSTEM II: CYTOCHROME B559, CHLOROPHYLL, AND β-CAROTENE
W. Rutherford1, P. Faller2 (1Gif-sur-Yvette, France, 2Freiburg, Germany)

IL006 APPLICATION OF A NEW NANOSECOND RESONANCE RAMAN TECHNIQUE IN THE STUDY OF PRIMARY CHARGE SEPARATION IN PHOTOSYSTEM II
A. Telfer1, L. Bordes2, J. Barber1, B. Robert1, A. Pascal2 (1London, United Kingdom, 2Gif sur Yvette, France)
Sunday, 7th September 2003

**FC007** SELF-ASSEMBLIES OF SYNTHETIC BACTERIOCHLOROPHYLL-D ANALOGUES POSSESsing A PORPHYRIN MOIETY  
H. Tamaki (Kusatsu, Japan)

**Room 5** Photoprotection: Sunscreens  
Chairs: A. Rougier (Asnières, France); B. Uhlmann (Hamburg, Germany)

**IL008** A WEIGHTING OF THE VARIABLES AFFECTING SPF MEASUREMENT  
B. L. Uhlmann (Hamburg, Germany)

**IL009** NEW INSIGHTS INTO THE DELETEROUS EFFECTS OF UVA-RADIATION AND DEVELOPMENT OF NOVEL PHOTOPROTECTIVE STRATEGIES  
J. Krutmann (Düsseldorf, Germany)

**IL010** A NEW BROADSPECTRUM SUNSCREEN IN THE PROTECTION OF UVA INDUCED PHOTODERMATOSES  
A. Rougier (Asnières, France)

**IL011** EVALUATION METHODS FOR SUNSCREENS IN THE UVA  
G. Leone (Rome, Italy)

**FC012** PHOTOPROTECTION OF SOME NATURAL FILTERS  
E. C. Fernandez³, F. Rancan², S. Rosan², K. Böhm², M. E. Hidalgo³, W. Quilhot³, C. Rubio³, F. Böhm², H. Piazena², U. Oltmanns² (¹Valparaiso, Chile, ²Berlin, Germany)

**FC013** ASSESSMENT OF THE BIOLOGICAL EFFECTS OF SOLAR AND UVA IRRADIATION AND OF THE EFFICIENCY OF PHOTOPROTECTION ON RECONSTITUTED HUMAN EPIDERMIS  
C. Gelis³, A. Mavon³, P. Vicendo² (¹Castanet Tolosan, France, ²Toulouse, France)

**FC014** UVA RADIATION INCREASES MELANOMA METASTASIS  
R. K. Pastila, D. Leszczynski (Helsinki, Finland)

**Room 7** DNA Damage and Repair  
Chairs: J. Cadet (Grenoble, France); A. Sarasin (Villejuif, France)

**IL015** DIRECT AND PHOTOSENSITIZED EFFECTS OF THE UV COMPONENTS OF SOLAR RADIATION ON CELLULAR DNA  
J. Cadet³, S. Cordavault³, J. Ravanat³, E. Sage², T. Douki² (¹Grenoble, France, ²Orsay, France)

**IL016** UVA-INDUCED DNA DAMAGE REVISITED  
E. Sage³, S. Kozmin³, A. Reynaud-Angelin³, T. Douki², J. Cadet³, P. J. Rochette³, R. Drouin³ (¹Orsay, France, ²Grenoble, France, ³Quebec, Canada)

**IL017** FORMATION AND REPAIR OF OXIDATIVE DNA DAMAGE INDUCED BY UV AND VISIBLE LIGHT  
S. Hoffmann¹, R. Greinert², B. Volkmer², B. Epe² (¹Mainz, Germany, ²Buxtehude, Germany)

**IL018** NUCLEOTIDE EXCISION REPAIR AND ITS INTERPLAY WITH TRANSCRIPTION  
L. Mullenders (Leiden, The Netherlands)

**IL019** BIOLOGICAL RESPONSE OF HUMAN PRIMARY KERATINOCYTES AND FIBROBLASTS TO UVB AND IONIZING RADIATION  
M. D’Errico², M. Teson², A. Calcagnile³, L. Proietti De Santis², M. Stefanini³, G. Zambruno³, E. Dogliotti³ (¹Rome, Italy, ²Viterbo, Italy, ³Pavia, Italy)
Sunday, 7th September 2003

**Room 8**

**Light Sources and Dosimetry in Medical Applications**
Chairs: S. Andersson-Engels (Lund, Sweden); L. Svaasand (Trondheim, Norway)

**IL023**
Non-ablative skin photo-rejuvenation
J. S. Nelson (Irvine, United States)

**IL024**
Tissue optical properties estimation for enhancing laser Doppler flowmetry
T. Strömberg (Linköping, Sweden)

**IL025**
Laser induced photothermolysis of port wine stains. Present state of the art and possible improvements
L. O. Svaasand (Trondheim, Norway)

**FC026**
Optoacoustic investigations of human skin in the UV
M. Meinhardt, R. Krebs, M. Bartels, A. Anders (Hanover, Germany)

**FC027**
Structural properties of GC polymers, DAPI complexed, studied by fluorescence correlation spectroscopy
M. L. Barcellona¹, E. Gratton² (¹Catania, Italy, ²Urbana, United States)

**FC028**
ALA-DYN: a non-coherent light source for ALA-PDT
T. Vidoczky, P. Baranyai (Budapest, Hungary)

**Room 9**

**Phototoxicity in the Eye**
Chairs: M. Boulton (Cardiff, United Kingdom); P. Söderberg (Stockholm, Sweden)

**IL029**
Transmittance of the eye
J. Dillon (New York, United States)

**IL030**
UV radiation effects on morphology and physiology of the lens
A. Wegener (Bonn, Germany)

**IL031**
In vivo ultraviolet radiation cataract
P. G. Söderberg, S. Löfgren, M. Ayala, M. Kakar, X. Dong, V. Mody (Stockholm, Sweden)

**FC035**
Maximum tolerable doses for cataract induced by UVR-B in age, exposure time and repeated exposure groups
X. Dong, P. G. Söderberg, S. Löfgren, M. Ayala, V. Mody, M. Kakar (Stockholm, Sweden)

**IL032**
The role of carotenoids in protection of unsaturated lipids against photoperoxidation
M. Wrona¹, A. Pawlak¹, M. Rozanowska², T. Sarna¹ (¹Krakow, Poland, ²Duke, United States)

**IL033**
The photoreactivity of ocular lipofuscin
M. E. Boulton, M. B. Rozanowska, B. Rozanowski, F. Shamsi (Cardiff, United Kingdom)

**L034**
Phosphatidylethanolamine prevents retinal photodamage
M. B. Rozanowska¹, B. Rozanowski², A. Pawlak¹, M. Zareba³, M. E. Boulton², T. Sarna¹ (¹Krakow, Poland, ²Cardiff, United Kingdom)
Sunday, 7th September 2003

**Room 4** 14.15 – 17.15

**SKIN PHOTOTYPES**
Chairs: A. Young (London, United Kingdom); N. Kollias (Skillman, United States)

**IL037** THE EXTENT TO WHICH VISIBLE „MELANIN“ MAY BE USED TO PREDICT SKIN REACTIVITY TO ULTRAVIOLET RADIATION
N. Kollias (Skillman, United States)

**IL038** PPF (PIGMENT PROTECTION FACTOR): AN OBJECTIVE MEASURE OF UVR SENSITIVITY AND ITS RELATION TO SKIN TYPE
P. A. Philipsen, E. Thieden, J. Sandby-Møller, H. Wulf (Copenhagen, Denmark)

**IL039** SKIN TYPE AND PIGMENT PROTECTION FACTOR: THE RELATIONSHIP BETWEEN SOLAR EXPOSURE BEHAVIOUR AND UVR EXPOSURE DOSE
E. Thieden, P. Philipsen, J. Sandby-Møller, H. Wulf (Copenhagen, Denmark)

**IL040** UV-INDUCED ERYTHEMA AND DNA DAMAGE IN DIFFERENT U.S. RACIAL/ETHNIC GROUPS
J. Z. Beer, V. J. Hearing, S. A. Miller, T. Tadokoro, Y. Yamaguchi, B. Z. Zmudzka (Rockville, United States, Bethesda, United States)

**IL041** SKIN TYPE AND APOPTOSIS
A. Young (London, United Kingdom)

**Room 5** PRECLINICAL PDT
Chairs: M. Korbelik (Vancouver, Canada); T. Patrice (Nantes, France)

**IL042** PDT SENSITIZERS APPROVALS: A NEED FOR A SPECIFIC CLASS OF DRUGS
T. J. Patrice, S. Thibault, F. Hudhomme, A. Furiga, Y. Lajat (Nantes, France)

**IL043** FLUENCE RATE DETERMINES THE INFLAMMATORY RESPONSE IN PHOTODYNAMIC THERAPY OF TUMORS
B. W. Henderson, S. O. Gollnick (Buffalo, United States)

**IL044** ACTIVATORS OF THE ALTERNATIVE COMPLEMENT PATHWAY ARE HIGHLY EFFICIENT ADJUVANTS TO PHOTODYNAMIC THERAPY FOR CANCER TREATMENT
M. Korbelik, J. Sun, I. Cecic, P. Cooper (Vancouver, Canada)

**FC045** USE OF ESR AND HET-CAM-ASSAY IN INVESTIGATION OF PHOTOTOXICITY
B. Algernissen, B. Jamil, A. Krink, D. Mangoldt, C. M. Philipp, H. Berlien (Berlin, Germany)

**FC046** SUPPRESSION OF CONTACT HYPERSENSITIVITY IN MICE BY PRODUCTS OF PROTOPORPHYRIN IX PHOTOOXIDATION
A. Y. Potapenko, A. A. Kyagova, G. V. Mansurova, L. A. Kozir, V. Y. Pavlov, I. O. Konstantinov, G. V. Ponomarev (Moscow, Russia)

**FC047** PHOTOREACTIVATORS AND PHOTOPRODUCTS IDENTIFICATION BY MASS SPECTROMETRY IN WHOLE CELLS
N. Lourette, B. Maunit, J. Muller, L. Bezdetnaya, F. Guillemin (Metz, France, Vandoeuvre les Nancy, France)
Room 8  **Laser-Tissue Interactions**  
Chairs: C. Edwards (Newport, United Kingdom); M. Landthaler (Regensburg, Germany)

**IL048**  **The Influence of Laser Parameters on the Treatment of Vascular Lesions**  
M. Landthaler (Regensburg, Germany)

**IL049**  **Selective Photothermolysis of Blood Vessels Using Indocyanine Green and Laser Irradiation**  
P. Babilas, V. Schacht, R. Engl, H. Stockmeier, W. Bäumler, R. Szeimies, C. Abels (Regensburg, Germany)

**IL051**  **Methods of Objective Assessment of Non-Ablative Skin-Laser Interactions.**  
C. Edwards (Newport, United Kingdom)

**IL052**  **Semi-Conductor Light Sources for Phototherapy and Photodiagnosis.**  
G. Jones (Swansea, United Kingdom)

**FC053**  **Pulsed Dye Laser Treatment of Sebaceous Gland Hyperplasia**  
M. M. Soliman (Guiza, Egypt)

**FC054**  **Histopathological Study of Xanthelasma Palpebarum After Pulsed Dye Laser Treatment**  
M. M. Soliman (Guiza, Egypt)

**IL055**  **Interaction of Laser and Tattoo Pigments**  
M. Landthaler (Regensburg, Germany)

Room 9  **Visual Pigments**  
Chairs: R. Crouch (Charleston, United States); D. Hunt (London, United Kingdom)

**IL056**  **Inner Retinal Photoreceptors in Mammals**  
R. J. Lucas¹, R. G. Foster¹, R. H. Douglas², M. W. Hankins³, S. Thompson³, S. Hattar², K. Yau²  
¹London, United Kingdom, ²Baltimore, United States

**IL057**  **Divergent Mechanisms for the Tuning of Shortwave Sensitive Visual Pigments in Vertebrates**  
D. M. Hunt (London, United Kingdom)

**IL058**  **Molecular Characterization of Visual Pigments in Aquatic Mammals**  
P. R. Robinson, J. I. Fasick, L. A. Newman (Baltimore, United States)

**IL059**  **The Molecular Mechanisms of Congenital Night Blindness**  
C. Cornwall (Boston, United States)

**IL060**  **Rod and Cone Photoreceptor Function in a Model Lacking 11-Cis Retinal**  
R. K. Crouch, J. Fan, S. Znoiko, J. Ma, B. Rohrer (Charleston, United States)

**FC061**  **Photodegradation Dynamics and Rates of Bacteriorhodopsin**  
D. M. Sammeth, J. M. McIntyre, J. R. Tafoya (Las Vegas, United States)
Monday, 8th September 2003

Room 1 09.00 – 09.45
PHOTOBIOLOGY UPDATE

IL062 ARE DIETARY CAROTENOIDS BENEFICIAL?
REACTIONS OF DIETARY CAROTENOIDS WITH OXY-RADICALS AND SINGLET OXYGEN
T. G. Truscott (Keele, United Kingdom) introduced by T. Sarna

09.45 – 10.15 Coffee Break

Room 4 10.15 – 13.15
STRATOSPHERIC OZONE AND THE LINK TO CLIMATE CHANGE
Chairs: R. L. McKenzie (Lauder, New Zealand); G. Seckmeyer (Hanover, Germany)

IL063 INTRODUCTION TO UV, OZONE AND CLIMATE CHANGE LINKS
R. L. McKenzie (Lauder, New Zealand)

IL064 STRATOSPHERIC OZONE AND CLIMATE CHANGE
L. Bengtsson (Hamburg, Germany)

IL065 OZONE/UV RELATIONS UNDER CHANGING FUTURE ATMOSPHERIC COMPOSITION
I. Isaksen (Oslo, Norway)

IL066 TOPICS OF INTEREST IN SOLAR UV MEASUREMENTS
G. Seckmeyer (Hanover, Germany)

FC067 UV RADIATION CLIMATOLOGY WITHIN THE SODA SYSTEM
A. R. Webb1, R. Kift1, J. Page1, S. Janjai2 (1Manchester, United Kingdom, 2Bangkok, Thailand)

FC068 AN ALGORITHM TO RETRIEVE BIOLOGICALLY WEIGHTED IRRADIANCE FROM IRRADIANCE MEASURED BY MULTI-CHANNEL RADIOMETERS
S. B. Diaz1, M. C. Carolina Camillion1, G. A. Deferrari2, C. E. Brunat1, C. R. Booth2, R. Armstrong3, S. Cabrera4, C. Cassiccia5, H. Fuenzalida4, C. Lovengreen6, A. Paladini7, J. Pedroni8, A. Rosales8, H. Zagarese9, M. Vernet2 (1Tierra del Fuego, Argentina, 2San Diego, United States, 3San Juan de Puerto Rico, United States, 4Santiago, Chile, 5Punta Arenas, Chile, 6Valdivia, Chile, 7Buenos Aires, Argentina, 8Trelew, Argentina, 9Bariloche, Argentina)

FC069 THE INFLUENCE OF MINI-OZONEHOLES TO THE BIOLOGICALLY EFFECTIVE ULTRAVIOLET RADIATION OVER CENTRAL EUROPE
A. Schmalwieser2, G. Schaubberger1, M. Janouch2 (1Vienna, Austria, 2Hradec Kralove, Czech Republic)

IL070 OZONE AND CLIMATE CHANGE EFFECTS ON PAST, PRESENT AND FUTURE UV-RADIATION LEVELS: CONSEQUENCES FOR SKIN CANCER RISKS
H. Slaper (De Bilt, The Netherlands)

Room 5 PHOTOAGEING
Chairs: P. Giacomoni (Melville, United States); F. Trautinger (Vienna, Austria)

IL071 LEARNING FROM THE PAST TO LOOK INTO THE NEXT WAVELENGTH
P. Giacomoni (Melville, United States)

IL072 SKIN AGING AND PHOTOAGING AS MODELS FOR ORGANISMIC AGING?
K. Scharffetter-Kochanek (Ulm, Germany)
Monday, 8th September 2003

**IL073** UV INDUCED CELLULAR RESPONSES IN SKIN: INTEGRATION OF RESPONSES TO GENOTOXIC STRESS WITH ENERGY METABOLISM AND MITOCHONDRIAL FUNCTION  
M. K. Jacobson (Tucson, United States)

**IL074** HEAT SHOCK PROTEINS IN PHOTOAGEING AND PHOTOCARCINOGENESIS  
F. Trautinger (Vienna, Austria)

**IL075** PHOTOAGING OF THE HUMAN EYE  
J. E. Roberts (New York, United States)

**FC076** OBJECTIVE ASSESSMENT OF PHOTOAGEING EFFECTS USING HIGH-FREQUENCY ULTRASOUND IN PUVA-TREATED PSORIASIS PATIENTS  
P. G. Sator, J. B. Schmidt, H. Höögsmann (Vienna, Austria)

**Room 8**  
Photosensory Biology  
Chairs: G. Checcucci (Pisa, Italy); W. Gärtner (Mülheim, Germany)

**IL077** BEHAVIORS PRODUCING PHOTODISPERAL IN STENTOR AND THEIR DEPENDENCE ON PHOTOTRANSDUCTION  
D. C. Wood (Pittsburgh, United States)

**IL078** TOWARDS A PROTEIN MAP OF THE GREEN ALGAL EYESPOT: ANALYSIS OF EYESPOT GLOBULE-ASSOCIATED PROTEINS  
G. Kreimer, S. Renninger (Erlangen, Germany)

**IL079** GENOMIC ANALYSIS OF THE SHADE AVOIDANCE RESPONSE  
P. F. Devlin, M. J. Yanovsky, S. A. Kay (London, United Kingdom, Buenos Aires, Argentina, La Jolla, United States)

**IL080** THE BACTERIAL COUNTERPARTS OF HIGHER PLANTS PHOTOTROPINS  
A. Losi (Parma, Italy)

**Room 9**  
Psoralens  
Chairs: F. Dall’Acqua (Padova, Italy); J. E. Hearst (Berkeley, United States)

**IL081** PSORALEN PHOTOCHEMOTHERAPY- FROM DAWN TO PRESENT DAY  
H. Höögsmann (Vienna, Austria)

**IL082** PHOTOCHEMICAL CROSSLINKING OF MICROBIAL GENOMES AS AN APPROACH TO DERIVE VACCINE PLATFORMS THAT COMBINE IMMUNOPOTENCY WITH SAFETY  
T. Dubensky (Concorde, United States)

**IL083** THE PIVotal ROLE OF MITOCHONDRIA IN PSORALEN-INDUCED APOPTOSIS  
M. Canton, S. Caffieri, F. Dall’Acqua, F. Di Lisa (Padova, Italy)

**IL084** PSORALEN FROM LAB BENCH, THROUGH CLINICAL TRIALS, TO THE MARKET  
J. E. Hearst (Berkeley, United States)

**FC085** PSORALEN–UV-A VS NARROWBAND UV-B PHOTOTHERAPY FOR THE TREATMENT OF VITILIGO  
D. Parsad, A. J. Kanwar, B. Kumar (Chandigarh, India)

**FC086** DNA DAMAGE AND BIOLOGICAL CONSEQUENCES INDUCED BY SOME ANGULAR FUROQUINOLINONES  
F. Bordin, F. Baccichetti, A. Chilin, F. Bettio, C. Marzano (Padua, Italy)

**FC087** PSORALEN PHOTOPRODUCTS WITH POSSIBLE APOPTOTIC ACTIVITY  
S. Caffieri, M. Canton, F. Di Lisa, F. Dall’Acqua (Padua, Italy)
Monday, 8th September 2003

**FC088** THE ROLE OF PHOTO-ACTIVATED PSORALENS IN THE CELL CYCLE
G. Viola, L. Facciolo, D. Vedaldi, F. Dall’Acqua, S. Disarò, G. Basso (Padua, Italy)

13.15 – 14.15 Lunch Break

**14.15 – 15.30**
**Poster Session I**

**Room 4** 15.30 – 18.30
**Photocarcinogenesis**
Chairs: F. R. de Gruijl, (Leiden, The Netherlands); H. Black (Houston, United States)

**IL089** PRO-AND ANTI-PHOTOCARCINOGENIC MECHANISMS OF ANTIOXIDANTS
H. S. Black (Houston, United States)

**IL090** TUMOR DEVELOPMENT IN HAIRLESS XPA INK4A KO MICE UNDER VARIOUS NEONATAL AND ADULT UV EXPOSURE REGIMENS
A. van Schanke¹, H. van Kranen², L. Mullenders¹, F. R. de Gruijl² (¹Leiden, The Netherlands, ²Bilthoven, The Netherlands)

**IL091** ROLE OF P53 AND FAS LIGAND IN PUVA-INDUCED APOPTOSIS AND CARCINOGENESIS
H. N. Ananthaswamy (Houston, United States)

**IL092** ACCUMULATION AND PERSISTENCE OF DNA DAMAGE IN PUTATIVE STEM CELLS IN MOUSE AND HUMAN EPIDERMIS
D. L. Mitchell², R. Greinert², E. Breitbart², B. Volkmer² (¹Smithville, United States, ²Buxtehude, Germany)

**IL093** THE LABORATORY OPOSSUM MODEL FOR UVB-INDUCED SKIN AND EYE CANCERS
J. L. VandeBerg (San Antonio, TX, United States)

**FC094** THE CONTRIBUTION OF CALPAINS IN THE DOWNREGULATION OF MDM2 AND P53 PROTEOLYSIS IN RECONSTRUCTED HUMAN EPIDERMIS IN RESPONSE TO SOLAR IRRADIATION
C. Gelis, A. Mavon, P. Vicendo (Toulouse, France)

**FC095** INDUCTION OF CPD RETAINING BASAL CELLS AFTER UV-IRRADIATION OF HUMAN SKIN – A FIRST STEP OF CANCER INITIATION?
B. Volkmer², S. Henning¹, D. L. Mitchell², E. W. Breitbart¹, R. Greinert¹ (¹Buxtehude, Germany, ²Smithville, United States)

**Room 5** 17.00 – 18.30
**Photodiagnosis**
Chairs: R. Baumgartner (Munich, Germany); G. Bottiroli (Pavia, Italy)

**IL096** IS FLUORESCENCE DIAGNOSIS WITH 5-ALA A BREAKTHROUGH IN INTRA-OPERATIVE CANCER DETECTION?
H. Stepp (Munich, Germany)

**IL097** HYPERICIN AS A TUMOUR DIAGNOSTIC TOOL
P. De Witte, G. Bornmans, A. Verbruggen (Leuven, Belgium)

**FC098** MACROSCOPIC AND MICROSCOPIC FLUORESCENCE IMAGING OF HUMAN BLADDER CANCER USING HYPERICIN AS A PHOTOSENSITIZER
M. C. Olivo, W. Lau, V. Manivasager, P. Tan, K. Soo, C. Cheng (Singapore, Singapore)
Monday, 8th September 2003

**FC099**  PHOTODYNAMIC DETECTION OF DISEASED SENTINEL LYMPH NODE AFTER ORAL APPLICATION OF AMINOLEVULINIC ACID (ALA) IN PATIENTS WITH BREAST CANCER

K. A. Frei1, H. M. Bone1, R. A. Steiner2, H. Walt3 (1Bern, Switzerland, 2Chur, Switzerland, 3Zurich, Switzerland)

**IL100**  AUTOFLUORESCENCE AS AN INTRINSIC PARAMETER FOR BIOLOGICAL TISSUE CHARACTERIZATION

G. Bottiroli (Pavia, Italy)

**IL101**  DETECTION OF PRECANCEROUS AND EARLY CANCEROUS LESIONS IN THE BRONCHI BY FLUORESCENCE/REFLECTANCE IMAGING WITH A SPECTRALLY OPTIMIZED SYSTEM

G. Wagnieres (Lausanne, Switzerland)

**FC102**  AUTOFLUORESCENCE OF TUMOUR TISSUE: PROSPECTS OF OPTICAL BIOPSY

M. Tamosiunas, J. Makaryceva, J. Labanauskiene, J. Didziapetriene, S. Bagdonas (Vilnius, Lithuania)

**FC103**  PREDICTING THE REACTION OF PHOTOSENSITIVE PATIENTS TO POLychROMATIC LIGHT SOURCES: A MATHEMATICAL METHOD BASED ON MONOCHROMATOR TESTING

H. E. Oliver1,2, H. Moseley1, J. Ferguson1 (1Dundee, United Kingdom, 2Uxbridge, United Kingdom)

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**Room 8**  REGULATION AND FUNCTION OF PLANT SECONDARY METABOLITES

Chairs: J. F. Bornman (Flakkebjerg, Denmark); G. Jenkins (Glasgow, United Kingdom)

**IL104**  REGULATION AND FUNCTION OF FLAVONOID BIOSYNTHESIS IN ARABIDOPSIS SEED


**IL105**  REGULATION OF FLAVONOID BIOSYNTHESIS GENES AND ASPECTS OF UV PROTECTION

G. I. Jenkins (Glasgow, United Kingdom)

**IL106**  UV R8 AND DIRECT UV-B SIGNAL TRANSDUCTION

D. J. Kliebenstein (Davis, United States)

**IL107**  NUTRITIONAL ENHANCEMENT OF PLANTS BY MANIPULATION OF SECONDARY METABOLITE LEVELS

M. Verhoeven (Bedford, United Kingdom)

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**Room 9**  NOVEL INSTRUMENTATION AND LASER SOURCES IN BIOLOGY AND MEDICINE

Chairs: H. Schneckenburger (Aalen, Germany); P. Taroni (Milan, Italy)

**IL108**  FLUORESCENCE CORRELATION SPECTROSCOPY UNRAVELS SIGNALING DYNAMICS IN THE APOPTOTIC PATHWAY

J. Wrachtrop (Stuttgart, Germany)

**IL109**  LASER-ASSISTED FLUORESCENCE MICROSCOPY IN CELL BIOLOGY AND PHOTOBIOLOGY

H. Schneckenburger1, M. Wagner1, M. Kretzschmar1, W. S. L. Strauss2, R. Sailer2 (1Aalen, Germany, 2Ulm, Germany)

**IL110**  FLUORESCENCE LIFETIME IMAGING

P. M. W. French (London, United Kingdom)

**FC111**  FLUORESCENCE LIFETIME IMAGING OF PHOTOSENSITIZER METABOLITES USING PS DIODE LASERS AND TIME-CORRELATED-SINGLE-PHOTON-COUNTING IN LASER SCANNING MICROSCOPES

A. Rück, F. Dolp, E. Haseroth, C. Scalfi-Happ (Ulm, Germany)

**IL112**  TIME-RESOLVED OPTICAL MAMMOGRAPHY: FROM INSTRUMENTATION DEVELOPMENT TO CLINICAL APPLICATION

P. Taroni, A. Pifferi, L. Spinelli, A. Torricelli, G. Danesini, R. Cubeddu (Milan, Italy)
Tuesday, 9th September 2003

Room 1 09.00 – 09.45
PHOTOBIOLOGY UPDATE

IL113 Photodermatology
T. Schwarz (Münster, Germany) introduced by H. Höngsman

09.45 – 10.15 Coffee Break

Room 4 10.15 – 13.15
ALA-BASED PDT
Chairs: A. Oseroff (Buffalo, United States); R.-M. Szeimies (Regensburg, Germany)

IL114 ALA AND ESTER-ALA-PDT – BASIC PRINCIPLES AND MOLECULAR MECHANISMS OF ACTION
B. Krammer, T. Verwanger, R. Sanovic (Salzburg, Austria)

IL115 CLINICAL EXPERIENCES WITH 5-ALA-PDT IN UROLOGY AND NEUROSURGERY
R. Baumgartner (Munich, Germany)

IL116 ALA AND ESTER-ALA-PDT FOR ONCOLOGIC INDICATIONS IN DERMATOLOGY
A. Oseroff (Buffalo, United States)

IL117 ALA-PDT FOR NON-ONCOLOGIC INDICATIONS IN DERMATOLOGY
R.-M. Szeimies (Regensburg, Germany)

FC118 5-AMINOLAEVULINIC ACID-CONTAINING DENDRIMERS AND OTHER DERIVATIVES AS PRODRUGS FOR PHOTODYNAMIC THERAPY: SYNTHESIS AND BIOLOGICAL EVALUATION
S. H. Battah1, H. Nakanishi2, P. Dobbin3, C. Edwards3, S. MacRobert1 (1London, United Kingdom, 2Tokushima, Japan, 3Cochester, Essex, United Kingdom)

FC119 HEXYL AMINOLEVULINATE (HAL) FOR DIAGNOSIS AND TREATMENT OF BLADDER CANCER
J. Afseth (Oslo, Norway)

Room 5 ENVIRONMENTAL PHOTOBIOLOGY
Chairs: S. Braslavsky (Mülheim, Germany); P. Rettberg (Cologne, Germany)

IL120 PAR AND UVBR INDUCED OXIDATIVE STRESS AND RELATED PROTECTION MECHANISMS IN ANTARCTIC MARINE MICROALGAE
A. G. Buma1, R. L. van den Enden2, S. W. Wright2, A. D. Davidson2 (1Haren, The Netherlands, 2Kingston, Australia)

IL121 BIOLOGICAL UV DOSIMETRY
P. Rettberg (Cologne, Germany)

IL122 HFR1, A PUTATIVE bHLH TRANSCRIPTION FACTOR, MEDIATES BOTH PHYTOCHROME A AND CRYPTOCHROME SIGNALLING
C. Fankhauser, P. D. Duek (Geneva, Switzerland)

IL123 BACTERIOPHYTOCHROMES AND REGULATION OF THE SYNTHESIS OF THE PHOTOSYNTHETIC APPARATUS IN RHODOPSEUDOMONAS PALUSTRIS
A. R. L. Vermeglio (Saint Paul lez Durance, France)

FC124 UV PROTECTION AND SHADE STRUCTURES
D. J. Turnbull, A. V. Parisi, J. Sabburg (Toowoomba, Australia)
Tuesday, 9th September 2003

**FC125** PHOTOCHEMISTRY AND PHOTOTOXICITY OF 1-AMINOPYRENE  
H. Yu, H. Hwang, K. Zeng, X. Shi (Jackson, United States)

**FC126** IMPACT OF RIBOFLAVIN ON PHOTO-TRANSFORMATION AND PHOTO-TOXICITY OF SELECTED ENVIRONMENTAL CONTAMINANTS IN WATER  
H. Hwang, K. Zeng, H. E. Glover (Jackson, United States)

**FC127** RED/FAR-RED ILLUMINATION AND LOW-FREQUENCY ELECTROMAGNETIC FIELDS INDUCE A STRESS EFFECT UPON HIGHER PLANTS, AS EVIDENT BY THE UNIVERSAL STRESS SIGNAL, ALANINE  
E. Monselise, A. H. Parola, D. Kost (Beer-Sheva, Israel)

**Room 8** PHOTOIMMUNOLOGY  
Chairs: T. Schwarz (Münster, Germany); M. Norval (Edinburgh, United Kingdom)

**IL128** ULTRAVIOLET RADIATION SUPPRESSION OF RECALL IMMUNITY IN HUMANS: NITRIC OXIDE, DNA DAMAGE AND UVA  
G. M. Halliday, J. M. Kuchel, T. S. C. Poon, S. N. Byrne, R. S. Barnetson (Sydney, Australia)

**IL129** UPDATE ON UV-INDUCED SUPPRESSOR/REGULATORY T CELLS  
Y. Aragane (Osakasayama-City, Japan)

**IL130** UVR-INDUCED IMMUNE MODULATION IN HUMANS – EFFECT OF SKIN TYPE  
S. L. Walker (London, United Kingdom)

**FC131** THE EFFECTS OF UV IRRADIATION WITH CLEO NATURAL SUNLAMPS ON INNATE IMMUNE PARAMETERS IN MICE  
P. McLoone, M. Norval (Edinburgh, United Kingdom)

**FC132** THE LIGHT-ACTIVATION OF HUMAN T-CELLS  
C. H. Self, A. C. Self, J. A. Smith, M. Fawcett, S. Thompson (Newcastle upon Tyne, United Kingdom)

**FC133** SYSTEMICALLY IMMUNOSUPPRESSIVE DOSES OF SOLAR-SIMULATED UV ACTIVATES LYMPH NODE B CELLS SO THAT THEY BECOME THE DOMINANT ANTIGEN PRESENTING CELL IN VIVO  
S. N. Byrne, N. Spinks, G. M. Halliday (Sydney, Australia)

**FC134** PROINFLAMMATORY PLATELET-ACTIVATING FACTOR (PAF) PATHWAY INVOLVED IN PSORALEN+UVA (PUVA-) INDUCED IMMUNE SUPPRESSION  
P. Wolf, D. X. Nghiem, J. P. Walterscheid, Y. Matsumura, N. Kazimi, P. Khaskina, H. N. Ananthaswamy, S. E. Ullrich (Graz, Austria, Houston, United States)

**FC135** THE EFFECT OF LOW DOSES UVA-1 RADIATION ON IMMUNOGLOBULIN PRODUCTION BY ACTIVATED B-LYMPHOCYTES  
M. C. Polderman, C. van Kooten, N. P. Smit, S. W. Kamerling, S. Pavel (Leiden, The Netherlands)

**FC136** PROLONGED SUSCEPTIBILITY TO LOCAL IMMUNOSUPPRESSION AFTER REPEATED NARROWBAND UVB VERSUS BROADBAND UVB CORRELATES WITH INCREASED INTRAEPIDERMAL CGRP  
F. J. Legat, P. Wolf, L. T. Jaiani, R. Lang, M. Wang, C. A. Armstrong, J. C. Ansel, J. D. Glass (Graz, Austria, Atlanta, United States, Chicago, United States)
Tuesday, 9th September 2003

FC137 REGULATION OF PHOTOIMMUNOSUPPRESSION – WHAT ABOUT ANTIOXIDANTS AND OESTROGEN?
V. E. Reeve, S. Widyarini, D. Titmuss (Sydney, NSW, Australia)

FC138 PERCUTANEOUS APPLICATION OF VISIBLE AND INFRARED LIGHT AT A THERAPEUTIC DOSE INDUCES CHANGES OF PRO- AND ANTI-INFLAMMATORY CYTOKINE CONTENT IN HUMAN
N. A. Zhevago, K. A. Samoilova, K. D. Obolenskaya (St. Petersburg, Russian Federation)

FC139 CIS-UROCANIC ACID STIMULATES NFkB ACTIVATION IN PRIMARY HUMAN KERATINOCYTES
U. Smetana-Just1, S. John1, M. Norval1, S. Walker1 (1London, United Kingdom, 2Edinburgh, United Kingdom)

Room 9 UV-induced Genomic Instability and Oxidation Induced by Ultraviolet Radiation
Chairs: E. Sage (Orsay, France); L. Mullenders (Leiden, The Netherlands)

IL140 TRANSCRIPTIONAL MUTAGENESIS IN PROKARYOTES AND EUKARYOTES
P. W. Doetsch (Atlanta, United States)

IL141 MECHANISMS OF UV-INDUCED MUTATIONS
G. Pfeifer (Duarte, United States)

IL142 MOLECULAR MECHANISMS OF UV-INDUCED MUTATIONS IN HUMAN CELLS PROFICIENT OR DEFICIENT IN DNA POLYMERASE ETA
A. Sarasin1, P. Kannouche2, A. Lehmann2, A. Stary1 (1Villejuif, France, 2Falmer, United Kingdom)

IL143 MAPK PATHWAYS AND AP-1 TRANSCRIPTION FACTORS IN DNA DAMAGE RESPONSES
H. van Dam (Leiden, The Netherlands)

IL144 EXPERIMENTS ON THE CAUSAL CHAIN FROM UV-INDUCED DANN DAMAGE AND REPAIR TO TUMOR FORMATION
F. de Gruijl (Leiden, The Netherlands)

13.15 – 14.15 Lunch Break

Room 4 14.15 – 17.15
Experimental PDT
Chairs: B. Ehrenberg (Ramat Gan, Israel); J. Piette (Liege, Belgium)

IL145 POTENTIATION OF PHOTODYNAMIC THERAPY WITH HYPERICIN BY MITOMYCIN C IN THE RIF-1 MOUSE TUMOR MODEL
P. De Witte (Leuven, Belgium)

IL146 ALA-PDT DEPENDENCE ON UP AND DOWN REGULATION OF THE PBGD GENE IN CANCER CELLS
Z. Malik (Ramat Gan, Israel)

IL147 COMBINATION REGIMENS WITH ALA-PDT
B. Ortel1, A. K. Sinha1, E. V. Maytin2, T. Hasan1 (1Boston, United States, 2Cleveland, United States)
Tuesday, 9th September 2003

IL148 A NEW APPROACH USING PORPHYRINS AS RADIOSENSITIZING AGENTS FOR SOLID TUMORS
(1Munich, Germany, 2Padua, Italy)

FC149 IS PDT GENERATED SINGLET OXYGEN LUMINESCENCE A PREDICTOR OF TREATMENT RESPONSE IN VITRO AND IN VIVO?
M. J. Niedre4, B. C. Wilson3, M. S. Patterson2 (1Toronto, Canada, 2Hamilton, Canada)

FC150 LYSOSOME TARGETING BY TETRA-CATIONIC PORPHYRINS
L. Franchi1, L. Borsetto1, G. Miotto1, F. Ricchelli1, P. Nikolov2, E. Reddi3 (1Padua, Italy, 2Sofia, Bulgaria)

FC151 PHOTOSENSITIZATION WITH ASYMMETRIC CHLORINS: SUBCELLULAR LOCALIZATION, EFFICACY AND APOPTOSIS INDUCTION IN JURKAT CELLS
F. Rancan, S. Al Omari, A. Wiehe, F. Böhm, B. Röder (Berlin, Germany)

FC152 MODULATION OF ADHESION MOLECULES EXPRESSION IN ENDOTHELIAL CELLS TREATED BY PHOTODYNAMIC THERAPY
C. Volanti, G. Gloire, Y. Habraken, J. Piette (Liege, Belgium)

Room 5  
JOINT ESP/EPA SYMPOSIUM ON CAROTENOIDS
Chairs: T. G. Truscott (Keele, United Kingdom); T. Gillbro (Umeå, Sweden)

IL153 CAROTENOIDS AND SUN PROTECTION
H. Sies, W. Stahl (Düsseldorf, Germany)

IL154 ENERGY TRANSFER DYNAMICS IN BIOLOGICAL AND ARTIFICIAL ANTENNA SYSTEMS CONTAINING CAROTENOIDS
T. Gillbro (Umeå, Sweden)

IL155 CAROTENOIDS PROTECT HUMAN SKIN CELLS IN VITRO AND PRIMATE EYES IN VIVO FROM DAMAGE BY UVA AND NEAR-VISIBLE RADIATIONS
R. Goralczyk2, J. Eicker3, M. Berneburg3, J. Krutmann2, M. Treklit4, R. M. Tyrrell4, G. Riss4, F. Barker5 (1Basel, Switzerland, 2Düsseldorf, Germany, 3Tübingen, Germany, 4Bath, United Kingdom, 5Philadelphia, United States)

IL156 HUMAN CELL PROTECTION BY CAROTENOIDS
F. Böhm (Berlin, Germany)

IL157 ROLE OF β-CAROTENE IN THE REACTION CENTRE OF PHOTOSYSTEM II
A. Telfer, J. Barber (London, United Kingdom)

FC158 ANTIOXIDANT ACTIVITY OF LYCOPENE EVALUATED IN TOPICAL APPLICATION AND IN VITRO
M. Andreassi, A. Ettorre, E. Stanghellini, A. Di Stefano, L. Andreassi (Siena, Italy)

FC159 ROLE OF CAROTENOIDS IN B800-B850 ENERGY TRANSFER IN THE LIGHT HARVESTING COMPLEX 2 (LH2) OF PURPLE BACTERIA
D. E. Leupold1, M. A. Krikunova1, 2, B. L. Voigt3, H. Lokstein3, H. Scheer4, A. A. Moskalenko5, A. P. Razjivin2 (1Berlin, Germany, 2Moscow, Russian Federation, 3Munich, Germany, 4Puschino, Russian Federation)
Room 8

**PHOTOMEDICINE**

Chairs: E. Hölzle (Oldenburg, Germany); J. Hawk (London, United Kingdom)

**IL160** PHOTOTOXIC SKIN REACTIONS – MECHANISMS, SCREENING PROCEDURES, DIAGNOSIS AND TREATMENT

J. Ferguson (Dundee, United Kingdom)

**IL161** SOLAR URTICARIA – PHOTOREACTION OF IMMEDIATE TYPE

E. Hölzle (Oldenburg, Germany)

**IL162** POLYMORPHIC LIGHT ERUPTION AND ACTINIC PRURIGO: NATURE, MECHANISMS, DIAGNOSIS AND TREATMENT

J. Hawk (London, United Kingdom)

**IL163** PHOTOPROTECTION – NEW DEVELOPMENTS

A. Young (London, United Kingdom)

**FC164** A PROSPECTIVE PLACEBO-CONTROLLED RANDOMISED DOUBLE-BLIND STUDY OF TETRACAINE GEL (AMETOP®) FOR PAIN RELIEF DURING TOPICAL PHOTODYNAMIC THERAPY

M. V. Holmes, R. S. Dawe, J. Ferguson, S. H. Ibbotson (Dundee, United Kingdom)

Room 9

**AQUATIC ECOSYSTEMS**

Chairs: M. Vernet (San Diego, United States); R. Smith (Santa Barbara, United States)

**IL165** UV EFFECTS ON THE MARINE PLANKTONIC FOOD WEB BASED ON MESOCOSM STUDIES

S. Roy (Rimouski, Canada)

**IL166** UV EFFECTS IN MARINE SYSTEMS

L. A. Franklin, P. J. Neale (Edgewater, United States)

**IL167** UV RADIATION IN FRESHWATER SYSTEMS: ITS EFFECTS ON ORGANISMS AND ECOSYSTEM PROCESSES

H. E. Zagarese (Chascomús, Argentina)

**FC168** EFFECTS OF UV-B RADIATION ON RICE-FIELD CYANOBACTERIA

R. P. Sinha, D. P. Häder (Erlangen, Germany)

**FC169** THE IMPACT OF UV RADIATION ON PLANKTONIC HETEROTROPHIC FLAGELLATES AND THEIR BACTERIVORY RATES

R. Sommaruga (Innsbruck, Austria)

Room 1

17.15 – 18.30

**GENERAL ASSEMBLY**

19.00

**DINNER AT A VIENNESE “HEURIGEN” & MEDAL AWARDS**
Wednesday, 10th September 2003

Room 1  09.00 – 09.45
PHOTOBIOLOGY UPDATE

IL170  SIGNALLING PATHWAYS REGULATING THE CELL DEATH AND SURVIVAL BALANCE IN RESPONSE TO PHOTODYNAMIC THERAPY WITH HYPERICIN
P. Agostinis (Leuven, Belgium) introduced by J. Piette

09.45 – 10.15 Coffee Break

Room 4  10.15 – 13.15
PDT – FREE COMMUNICATIONS
Chairs: S. B. Brown (Leeds, United Kingdom); E. Reddi (Padua, Italy)

FC 171  PHYSICAL PROPERTIES OF MEMBRANES AFFECT THE UPTAKE, TOPOGRAPHY AND EFFICIENCY OF PHOTOSENSITIZERS
B. Ehrenberg¹, I. Bronshtein¹, M. Kepczynski¹, A. Lavi³, K. M. Smith² (¹Ramat Gan, Israel, ²Baton Rouge, United States)

FC 172  FLUORESCENCE LIFETIME IMAGING AND SPECTRAL BEHAVIOUR OF PROTOPORPHYRIN IX (PPIX) IN HUMAN TUMOR CELLS – CORRELATION WITH PHOTODYNAMIC INACTIVATION
R. Sailer³, W. Strauss³, M. Wagner², M. Kretzschmar², R. Steiner¹, H. Schneckenburger¹² (¹Ulum, Germany, ²Aalen, Germany)

FC 173  RELATIONSHIP BETWEEN MTHPC PHOTOBLEACHING AND CELL VIABILITY DURING IN VITRO PHOTODYNAMIC THERAPY
J. S. Dysart, M. S. Patterson, G. Singh (Hamilton, Canada)

FC 174  EFFECTS OF POLYUNSATURATED FATTY ACIDS ON 5-AMINOLEVULINIC ACID BASED PHOTOSENSITISATION IN FOUR DIFFERENT HUMAN CANCER CELL LINES
O. Gederaas, S. A. Schenberg, S. Ramstad, A. Johnsson, H. Krokan (Trondheim, Norway)

FC 175  PHOTODEGRADATION AND PHOTOTRANSFORMATION OF 5,10,15,20-TETRAKIS(M-HYDROXYPHENYL)BACTERIOCHLORIN (M-THPBC) IN SOLUTION
H. P. Lassalle¹², L. Bezdetnaya¹, F. Guillemín¹, J. Moan² (¹Vandoeuvre-les-Nancy, France, ²Oslo, Norway)

FC 176  PDT EFFECTS ON THE ADHESIVE PROPERTIES OF CULTURED HUMAN CARCINOMA AND GLIOMA CELLS
A. B. Uzdensky¹², A. Juseniene², J. Moan² (¹Rostov-on-Don, Russian Federation, ²Oslo, Norway)

FC 177  HOW DO METHYLENE BLUE DERIVATIVES KILL V79 CELLS DURING PHOTODYNAMIC THERAPY?
R. K. Tyler, S. L. Hankin, S. A. Gorman, D. I. Vernon, J. Griffiths, S. B. Brown (Leeds, United Kingdom)

FC 178  DEVELOPMENT OF A TEST SYSTEM FOR MUTAGENICITY OF PHOTOSENSITIZERS USING DROSOPHILA MELANOGASTER

FC 179  COMPLEXES OF ADENOVIRUS WITH POLYCATIONS INCREASE THE EFFICIENCY OF PHOTOCHEMICALLY MEDIATED TRANSDUCTION
A. Bonsted, B. O. Engesaeter, A. Hogset, G. M. Maelandsmo, L. Prasmickaitė, O. Kaalhus, K. Berg (Oslo, Norway)
Wednesday, 10th September 2003

**Room 5**

**Photochemistry/ Phototoxicity of Drugs**

**FC180** TISSUE DETECTION OF DIPHENYLCHLORIN SENSITIZER (SIM01) BY FLUORESCENCE AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY  
S. Thibaut, L. Bourré, M. Fimiani, Y. Lajat, T. Patrice (Nantes, France)

**FC181** PHOTODYNAMIC THERAPY AND FLUORESCENT DIAGNOSTICS OF BREAST CANCER WITH PHOTOSENSE  
E. G. Vakulovskaya, L. V. Oumnova, G. N. Vorozshcov (Moscow, Russian Federation)

**FC182** SYNERGISM OF ALA-PDT AND METHOTREXATE  
B. Ortel, A. Sinha, T. Hasan, E. Maytin (Boston, United States)

**IL183** PUTTING IN VITRO PHOTOTOXICITY TESTING INTO PRACTICE: THE IMPACT OF COMMENTS FROM OECD MEMBER COUNTRIES ON A SCIENTIFICALLY VALIDATED METHOD  
M. Liebsch (Berlin, Germany)

**IL184** PHOTOSENSITIZATION OF THYMINE NUCLEOBASE BY BENZOPHENONE DERIVATIVES AS MODELS FOR PHOTOSTIMULATED DNA DAMAGE  
S. Encinas, N. Belmadoui, M. J. Climent, M. A. Miranda (Valencia, Spain)

**IL185** SOME ASPECTS OF THE PHOTOTOXICITY INDUCED BY ANTIBACTERIAL FLUOROQUINOLONES IN NATIVE AND CELL DNA  
G. De Guidi (Catania, Italy)

**IL186** PHOTOTOXIC REACTIONS OF ISOLATED AND CELLULAR DNA  
J. Cadet, T. Douki, J. Ravanat, S. Sauvaigo (Grenoble, France)

**IL187** FLUOROQUINOLONE PHOTOTOXICITY IN SKIN TUMORIGENESIS  
N. K. Gibbs (Manchester, United Kingdom)

**IL188** NEW ASPECTS OF NSAID PHOTOTOXICITY  
J. Castell (Valencia, Spain)

**FC189** THE IN VITRO CHARACTERISATION OF A SERIES OF ASYMMETRIC PHENOTHIAZINIUM SALTS  
I. Walker, S. A. Gorman, D. I. Vernon, J. Griffiths, S. B. Brown (Leeds, United Kingdom)

**Room 8**

**Systemic Photoprotection**  
Chairs: L. E. Rhodes (Manchester, United Kingdom); H. Black (Houston, United States)

**IL190** SELENIUM AUGMENTS IMMUNITY AND RESISTANCE TO OXIDATIVE STRESS – A ROLE IN PHOTOPROTECTION?  
R. C. McKenzie (Edinburgh, United Kingdom)

**IL191** THE GENERATION OF MICRONUCELEI BY SOLAR RADIATION IN PRIMARY KERATINOCYTES IS INHIBITED BY EICOSAPENTENOIC ACID  
Eckert¹, M. Homburg¹, M. Garmyn², B. Epe¹ (¹Mainz, Germany, ²Leuven, Belgium)

**IL192** VITAMIN E REDUCES THE LIPID PEROXIDATION ASSOCIATED WITH EPA SUPPLEMENTATION AND ENHANCES RESISTANCE TO UVR-INDUCED ERYTHEMA IN HUMAN SKIN  
L. E. Rhodes³, R. M. W. Moison², G. M. J. Beijersbergen van Henegouwen²; (³Manchester, United Kingdom, ²Leiden, The Netherlands)

**IL193** PRO-CARCINOGENIC ACTIVITY OF BETA-CAROTENE, A PUTATIVE SYSTEMIC PHOTOPROTECTANT  
H. S. Black (Houston, United States)
Wednesday, 10th September 2003

**IL194**  HEAT SHOCK PROTEINS AND POTENTIAL HAZARDS OF EXTRINSIC PHOTOPROTECTION  
C. Jantschitsch, F. Trautinger (Vienna, Austria)

**FC195**  THE EFFECT OF TEA CATECHINS AND THEAFLAVINS ON UVB-INDUCED CYCLOOXYGENASE-2 PROTEIN EXPRESSION IN HaCaT KERATINOCYTES  
K. Uprichard, N. J. Traynor, H. Moseley, J. A. Woods (Dundee, United Kingdom)

**FC196**  NEW DEVELOPMENT IN PHOTOPROTECTION: ORAL SUPPLEMENTATION IN HEALTHY VOLUNTEERS PREVENTS THE OXIDATIVE, INFLAMMATORY AND IMMUNE UV-RESPONSES IN BLOOD CELLS  
C. Baudouin, M. Haure, C. Vaissiere, M. Aries, M. Charveron (Toulouse, France)

**Room 9**  PHOTOSYNTHESIS: ACCLIMATION, STRESS AND REGULATION  
Chairs: E.-M. Aro (Turku, Finland); U. Sonnewald (Gatersleben, Germany)

**IL197**  SINGLET OXYGEN AND THE ACTIVATION OF A GENETICALLY CONTROLLED SUICIDE PROGRAM IN ARABIDOPSIS THALIANA  
K. Apel, C. Laloi, A. Danon (Zurich, Switzerland)

**IL198**  INDUCIBLE RNAI OF CHLOROPHYLL BIOSYNTHETIC GENES IN TRANSGENIC TOBACCO PLANTS  
F. Börnke, U. Sonnewald, S. Chen (Gatersleben, Germany)

**IL199**  REPROGRAMMING LEAF METABOLISM BY PATHOGENS  
U. Sonnewald, H. Tschiersch, E. Glickmann, F. Börnke, S. Biemelt (Gatersleben, Germany)

**IL200**  TETRAPYRROLE INDUCED PHOTOSENSITIZATION IN PHOTOSYNTHETIC ORGANISMS  
B. C. Grimm (Berlin, Germany)

**IL201**  REVERSE GENETICS OF THE PLANT LIGHT HARVESTING ANTENNA  
S. Jansson¹, J. Andersson², U. Ganeteg¹, C. Külheim¹, E. Boekema², J. Dekker³, P. Horton⁴, J. Ågren² (¹Umeå, Sweden, ²Groningen, The Netherlands, ³Amsterdam, The Netherlands, ⁴Sheffield, United Kingdom, ⁵Uppsala, Sweden)

**IL202**  REDOX REGULATION OF THYLAKOID PROTEIN PHOSPHORYLATION  
E. Aro, M. Piippo, Y. Allahverdiyeva, N. Battchikova, E. Rintamäki (Turku, Finland)

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13.15 – 14.15 Lunch Break

14.15 – 15.30  
Poster Session II

**Room 4**  15.30 – 18.30  
ROLE OF ROS IN PHOTOBIOLOGY  
Chairs: É. Hideg (Szeged, Hungary); R. M. Tyrrell (Bath, United Kingdom)

**IL203**  ROLE OF CHLOROPLAST DERIVED ROS IN PLANT DEFENSE  
T. E. Palva, T. Kariola, J. Li, G. Brader (Helsinki, Finland)

**IL204**  ROS TURNOVER AND OXIDATIVE DAMAGE TO PROTEINS IN PLANT MITOCHONDRIA  
I. M. Möller (Roskilde, Denmark)

**IL205**  DETECTING REACTIVE OXYGEN SPECIES IN LEAVES STRESSED BY ULTRAVIOLET- OR EXCESS PHOTOSYNTHETICALLY ACTIVE RADIATION  
É. Hideg (Szeged, Hungary)
Wednesday, 10th September 2003

IL206 REACTIVE OXYGEN SPECIES GENERATED BY UVA IN MAMMALIAN CELLS – THE ROLE OF IRON
R. M. Tyrrell, C. A. Pourzand, P. Holley, J. L. Zhong (Bath, United Kingdom)

IL207 CYTOPROTECTIVE AND CYTOTOXIC EFFECTS OF IN VIVO FLAVONOID METABOLITES IN HUMAN SKIN FIBROBLASTS UNDER OXIDATIVE STRESS
C. Rice-Evans (London, United Kingdom)

FC208 OXIDATIVE DAMAGE AND CYTOTOXICITY OF UVA DEPENDS ON THE MODE OF EXPOSURE
H. Merwald, G. Klosner, H. Hönigsmann, F. Trautinger (Vienna, Austria)

FC209 DETECTION OF REACTIVE OXYGEN SPECIES BASED ON CHEMILUMINESCENCE OF LUMINOL AND CYPRIDINA LUCIFERIN ANALOGUES
M. Benciňová, I. Šnyrchová (Olomouc, Czech Republic)

FC210 LASER-INDUCED OXYGEN PHOSPHORESCENCE IN WATER; DEPENDENCE ON DETERGENTS AND SODIUM AZIDE
A. A. Krasnovsky, D. Butorina (Moscow, Russian Federation)

Room 5 DNA DAMAGE AND REPAIR – FREE COMMUNICATIONS
Chair: J. Piette (Liege, Belgium)

FC211 SUNLIGHT FORMS CYCLOBUTANE PYRIMIDINE DIMER, BUT NOT (6-4) PHOTOPRODUCT NOR 8-HYDROXYDEOXYGUANOSINE IN BROAD BEAN LEAVES
T. Hashimoto (Kobe, Japan)

FC212 FORMATION OF INTER-STRAND BIPYRIMIDINE PHOTOPRODUCTS WITHIN UVC-IRRADIATED DNA
T. Douki, J. Cadet (Grenoble, France)

FC213 A TOOL FOR THYMINE DIMERS PHOTOSENSITIZATION
J. Trzcionka1, V. Lhiaubet-Vallet2, N. Chouini-Lalanne1 (1Toulouse, France, 2Valencia, Spain)

FC214 HOW SUPEROXIDE DISMUTASE AFFECTS OXIDATIVE DAMAGE PHOTINDUCED ON DNA BY RU(BIPY)32+ AND RU(BPZ)32+. PROPOSAL OF A MECHANISM
E. Gicquel, J. Trzcionka, P. Vicendó (Toulouse, France)

FC215 NON-LETHAL DNA DAMAGE IN NON-MELANOMA SKIN CANCER CELLS INDUCED BY PHOTOFIN-PDT: A COMET ASSAY STUDY
J. A. Woods, N. J. Traynor, H. Moseley (Dundee, United Kingdom)

FC216 ROLE OF UV(A) INDUCED DNA DOUBLE-STRAND BREAKS (DNA-DSB) AS PRECURSOR LESIONS OF CHROMOSOMAL ABERRATIONS IMPORTANT FOR SKIN CANCER DEVELOPMENT
R. Greiner1, A. Rapp2, S. Henning1, B. Volkmer1 (1Buxtehude, Germany, 2Jena, Germany)

FC217 UVA IRRADIATION INHIBITS THE EXPRESSION AND SECRETION OF THE GELATINASES MMP-2 AND MMP-9 IN CULTURED PRIMARY HUMAN KERATINOCYTES
H. Steinbrenner2, M. C. Ramos3, D. Stuhlmann1, H. Sies1, P. Brenneisen1 (1Düsseldorf, Germany, 2Santo Tomas, Philippines)

Room 8 COSMETIC TANNING
Chair: P. Calzavara-Pinton (Brescia, Italy)

IL218 CELLULAR-MOLECULAR EFFECTS OF UVA
B. Ortel (Boston, United States)
IL219 IMMUNOLOGICAL EFFECTS OF ACUTE AND CHRONIC UVA EXPOSURES
S. Di Nuzzo (Parma, Italy)

IL220 BIOLOGICAL EFFECTS OF UVA/ UVB CONTENTS OF SUNBEDS
P. Calzavara Pinton, R. Sala, M. Venturini (Brescia, Italy)

IL221 UVA SUNBEDS IN THE TREATMENT OF SKIN DISEASES
H. Moseley (Dundee, United Kingdom)

IL222 VITAMIN D AND CANCER PROTECTION
J. Moan (Oslo, Norway)

FC223 THYMIDINE DINUCLEOTIDE: A NEW DNA PHOTO PROTECTOR FOR HUMAN SKIN
A. F. El Bedewi, S. S. Koraa, S. A. Abu Nour, D. A. Goukassian, B. A. Gilchrest;
(1Cairo, Egypt, 2Boston, United States)

Room 9 NATURAL PHOTORECEPTORS AND ARTIFICIAL PHOTODEVICES
Chairs: N. Hampp (Marburg, Germany); F. Lenci (Pisa, Italy)

IL224 INTRODUCTORY REMARKS
F. Lenci (Pisa, Italy)

IL225 EARLY MOLECULAR PROCESSES IN THE PHOTOACTIVE YELLOW PROTEIN: ROLE OF THE
CHROMOPHORE PHOTOPHYSICS
P. Changenet-Barret, A. Espagne, P. Plaza, M. M. Martin, S. Charier, J. Baudin, L. Jullien, K. Hellingwerf
(1Paris, France, 2Amsterdam, The Netherlands)

IL226 APPLICATIONS OF BACTERIORHODOPSIN IN OPTICAL INFORMATION PROCESSING
N. A. Hampp (Marburg, Germany)

IL227 OPTICAL DATA STORAGE USING PEPTIDES
R. H. Berg, P. S. Ramanujam (Roskilde, Denmark)

IL228 CONTROL OF ENERGY AND ELECTRON FLOW IN ARTIFICIAL PHOTOSYNTHETIC
ANTENNAS AND REACTION CENTERS
T. A. Moore, A. L. Moore, D. Gust (Tempe, United States)
Thursday, 11th September 2003

Room 1 09.00 – 09.45
PHOTOBIOLOGY UPDATE

IL229 ENVIRONMENTAL PHOTOBIOLOGY – IMPACTS OF OZONE DEPLETION AND CLIMATE CHANGE
R. L. McKenzie (Lauder, New Zealand) introduced by J. C. van der Leun

09.45 – 10.15 Coffee Break

Room 4 10.15 – 13.15
UV PHOTOTHERAPY
Chairs: H. Hönigsmann (Vienna, Austria); J. Ferguson (Dundee, United Kingdom)

IL230 NARROWBAND UVB (TL-01)
J. Ferguson (Dundee, United Kingdom)

IL231 PUVA
H. Hönigsmann (Vienna, Austria)

IL232 UVA1
A. Tanew (Vienna, Austria)

FC233 EXPRESSION AND REGULATION OF CYTOCHROME P450 CYP2S1 IN HUMAN SKIN BY ULTRAVIOLET RADIATION AND THERAPEUTIC AGENTS FOR PSORIASIS
G. Smith, R. Wolf, Y. Y. Deeni, R. S. Dawe, A. T. Evans, M. M. Comrie, J. Ferguson, S. H. Ibbotson (Dundee, United Kingdom)

FC234 THE PHOTOCARCINOGENIC RISK OF NARROWBAND TL-01 UVB PHOTOTHERAPY: EARLY FOLLOW UP DATA
I. Man (Dundee, United Kingdom)

FC235 EXCIMER 308 NM LASER PHOTOTHERAPY OF PSORIASIS. RESULTS OF MONOTHERAPY COMARED TO COMBINATION WITH CALCITRIOL
K. Fritz, B. Moos (Landau, Germany)

FC236 RANDOMIZED, DOUBLE-BLIND COMPARISON OF 0.0001% VS. 0.0005% METHOXSALEN BATH PUVA THERAPY FOR CHRONIC PLAQUE TYPE PSORIASIS
R. Vongthongsri, H. Hönigsmann, A. Tanew (Vienna, Austria)

FC237 LONG-TERM EFFICACY OF DERMODYNE UV-FREE PHOTOTHERAPY IN CHRONIC HAND AND FOOT DERMATITIS
J. H. Wilkens¹, K. Medve-Königs², N. Mahnke³, J. Krutmann² (¹Wilgau, Germany, ²Düsseldorf, Germany)

FC238 ZINC OCTA-DECYL PHTHALOCYANINE: A CANDIDATE FOR PHOTODYNAMIC TREATMENT OF PSORIASIS
L. Kaestner², M. Cesson³, K. Kassab³, T. Christensen², P. D. Edminson¹, M. J. Cook⁴, I. Chambrier⁴, G. Jori³; (¹Rykkinn, Norway, ²Østerås, Norway, ³Padova, Italy, ⁴Norfolk, United Kingdom)

Room 5 THE DIGITAL PHOTOBIOLOGY COMPENDIUM
Chairs: D. P. Valenzano, (Kansas City, United States); F. Lenci (Pisa, Italy)

IL239 THE DIGITAL PHOTOBIOLOGY COMPENDIUM: OVERVIEW AND EVALUATION
D. P. Valenzano (Kansas City, United States)

IL240 VIRTUAL SINGLET OXYGEN LUMINESCENCE MEASUREMENT
S. Nonell (Barcelona, Spain)

IL241 PHOTOSENSITIZATION OF SUBCELLULAR STRUCTURES
T. Christensen (Østerås, Norway)
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Y. Posudin, N. Massjuk, G. Lilitskaya (Kiev, Ukraine)

P002
Why Aquatic Bacterial Isolates Show Different Sensitivity to UV Radiation?
J. S. Hofer, R. Sommaruga (Innsbruck, Austria)

P003
(+), S-ABA and (-), R-ABA do not play any signaling role in the plants: they are stable end products from breakdown of carotenoids under stressful conditions
B. A. Kurchii (Kiev, Ukraine)

P004
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A. Emanuelsson, A. Szilágyi, H.-E. Åkerlund (Lund, Sweden)

P005
Measurement of erythema-effective irradiance and determination of skin type as conditions for a responsible use of solaria
D. Kockott¹, E. Menzel¹, R. Sippel³ (¹Hanau, Germany, ²Dortmund, Germany, ³Fröndenberg, Germany)

P006
Analysis of the genotoxic effect of combined UVB and UVA irradiation of human keratinocytes: Evaluation of the protective capacity of a sunscreen
M.-J. Haure¹, C. Baudouin¹, T. Douki², J. Cadet², A. Favier², M. Charveron² (¹Toulouse, France, ²Grenoble, France)

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A. Tarozzi, A. Marchesi, G. Cantelli Forti, P. Hrelia (*Bologna, Italy*)
Targeting of the vascular system of solid tumors by Photodynamic Therapy
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Due to morphologic and rheologic differences of the tumor vasculature as compared to the vascular system of the surrounding tissue the efficacy of several experimental and clinical therapeutic approaches is limited. This fact has put the vascular system of solid tumors into focus and two new therapeutic strategies, photodynamic tumor targeting and vascular targeting, have emerged. Under the term vascular targeting various therapeutic approaches are summarized, e.g. chemooembolisation, chemotherapy, hyperthermia, anti-endothelial antibodies and PDT. As shown with the clinically approved photosensitizer Photofrin® the irreversible destruction of the tumor vasculature is primarily responsible for an effective PDT of solid tumors. However, the clinical disadvantages of Photofrin® are well known. Thus, several new photosensitizers, ALA, phophyrines, ICG have been evaluated in vitro and in vivo regarding their suitability for vascular targeting of solid tumors. The promising experimental findings with the photosensitizer ICG lead to first clinical results in treating Kaposi sarcoma. In summary, only an effective, systematic PDT leads to complete ischemia of solid tumors thus yielding necrosis. An essential prerequisite is the use of a chemically and photophysiologically defined photosensitizer exhibiting a high molecular weight. The specific properties of such a photosensitizer are outlined.

Mimicking the Photosynthetic Functions of Carotenoid Polymers
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Carotenoid pigments incorporated into tetrapyrrole-based supramolecular ensembles mimic the role of these pigments in natural photosynthesis as antennas, as switches dissipating excess excitation energy, as molecular wires connecting redox centers, and as photoprotective agents. We have found precise structural, electronic, and thermodynamic parameters necessary for ~100-fold efficient carotenoid antenna function. As in the natural systems, high efficiency requires energy transfer from multiple carotenoid excited states acting over time scales from ~40 fs to ~20 ps. The attached carotenoid pigments quench effectively the tetrapyrrole excited singlet state in polar solvent by direct energy transfer from the carotenoid to the tetrapyrrole by the prominent transient absorption observed in the near IR, from ~850–1000 nm depending on the number or conjugated double bonds, which is characteristic of carotenoid radical cation species. In gold-containing carotenotetrapyrroles triplet-triplet energy transfer rates approaching 1012 s-1 have been measured establishing that superequilibrium mediated processes can occur on the picosecond time scale. In some reaction center mimics the charge recombination process is dominated by population of the carotenoid triplet excited state rather than recovery to the singlet ground state. This is not a common observation in artificial photosynthetic systems, but observed in natural reaction centers.

How plants see blue light: insights from time-resolved spectroscopy
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The phototropins constitute an important class of plant photoreceptor kinases that control a range of physiological responses including phototropism, light-directed chloroplast movement and stomatal opening. The LOV2 domain of phototropin binds a molecule of flavin mononucleotide (FMN) and undergoes a photocycle involving light-driven covalent adduct formation between a conserved cysteine residue and the C(4a) atom of FMN. Here, we report the primary photophysics and photochemistry of LOV2 domains of phototropin 1 of Avena sativa (oat) and of the phy3 photoreceptor of Adiantum capillus-veneris (maidenhair fern). We demonstrate that the FMN triplet state is the primary photoprotein in the LOV2 photocycle, generated at 60% efficiency. No spectroscopically distinguishable intermediates precede the FMN triplet on the femtosecond to nanosecond timescale, indicating that it is formed directly via intersystem crossing (ISC) from the singlet state. Our results indicate that the majority of the FMN triplets in LOV2 exist in the protonated form. We propose a reaction mechanism that involves excited-state proton transfer, on the nanosecond timescale or faster, from the sulfhydryl group of the conserved cysteine to the N5 atom of FMN. This event promotes adduct formation by increasing the electrophilicity of C(4a) and subsequent nucleophilic attack by the cysteine’s thiolate anion. Comparison to free FMN in solution shows that the protein environment of LOV2 increases the ISC rate of FMN by a factor of 2.4, thus improving the yield of the cysteinyl-flavin adduct and the efficiency of photosynthetic-mediated signaling processes. Upon excitation of the photocumulated adduct state with near-UV light, we find that the adduct is broken on a picosecond to nanosecond timescale. This indicates that the singling state of the LOVDomain can be switched on and off by light, and that LOV domains may have a 2-photon dependent function.

Functions of carotenoids and other pigments in photosystem II from a structural point of view
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Carotenoids play important roles in energy transfer and secondary electron transfer in photosystem II (PSII). It has long been reported that isolated PSII reaction center (RC) contains two molecules of β-carotenes. Our recent crystal structural analysis of PSII from the thermophilic cyanobacterium Thermosynechococcus vulcanus revealed two regions of electron density in the vicinity of PSII-RC that could be attributed to two molecules of carotenoids (1). Although the density for one of the two carotenoids is relatively weak and should be considered tentative, these carotenoids are located in a cavity surrounded by D1, D2, CP43 and cytochrome b–559, and are in a relatively close location with each other. The arrangement of these putative carotenoids yields important functional consequences on the energy and secondary electron transfer within PSII. These functional consequences will be discussed together with the arrangement of other pigment cofactors based on the recently obtained electron density map. (2) Kamiya N. and Shen J.-R. (2003) Proc. Natl. Acad. Sci. USA, 100, 98-103.

Side-Path Electron Donors in Photosystem II: Cytochrome b559, Chlorophyll, and β-Carotene
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β-Carotene, cytochrome b559 and a monomeric chlorophyll, designated Chl b, undergo oxidation in PSII under some illumination conditions. These components are not part of the electron transfer chain but lead to water oxidation and plastoquinone reduction, i.e. they are not directly involved in enzyme function. They thus designated “side-path electron donors.” Under the usual conditions of PSII function the quantum yield for the oxidation of these side path components is limited; however under experimental conditions used for studying PSII, particularly low temperatures the dominant electron donor can be those involving the side-path donors. β-carotene is a branch point in the side-path electron donation, being oxidized by P (the kineticly competent chlorophyll cation), and reduced by cytochrome b559 (or Chl b, when the cytochrome is pre-oxidised). A good deal of evidence and argument point to this occurring on the D2 side of the reaction center. Related reactions may occur on the D1 side but this is less clearly demonstrated and it remains possible that the side-path donors are purely D2-side species. Based on the low quantum yield, we argue that it is unlikely that the side-path donation pathway functions as a relevant photoprotective cycle aimed at removing low-living P and thus minimizing oxidative damage.

Application of a new nanosecond resonance Raman technique in the study of primary charge separation in photosystem II
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The identity and electronic structure of the chlorophylls bearing the radical cation of the primary electron donor, P680+, in the photosystem II reaction center are still under debate. We have compared continuously excited resonant Raman spectra from isolated PSII RCs with RR spectra obtained using 10 ns flashes. Continuous light at 413.1 nm gives rise to modes from all of the neutral pigments bound to the reaction center: chlorophylls, phyto- phyllins, beta-carotenes and cytochrome b559. Flash excitation (10 Hz) at the same wavelength excites charge separation to form P680+ Pheo, which decays with a half time of ~30 ns. Hence during the lifetime of the flash the yield of the radical pair is high (~80%). The RR modes of the neutral species carrying the charge-separated state are thus considerably reduced in size. Continuous-minus-ns spectra reveal the modes arising from the charge-bearing molecules. Analysis of these modes yields information on (i) the number of molecules involved in the charge-separated state (ii) their conformation and the nature of their interactions with the surrounding protein. This technique avoids the problem of secondary oxidation of chlorophyll and beta-carotene, which has previously hampered understanding of data obtained when P680+ is photocumulated.
FC007 Self-assemblies of synthetic bacteriochlorophyll-d analogues possessing a porphyrin moiety
H. Tamaki; 1Nishigaki University, Kuratsu, Japan.

IL008 A weighting of the variables affecting SPF measurement
R.L. Uhlmann; 1Bielefeld, Germany, 2Humboldt University, Berlin, Germany, 3Meoclinc, Berlin, Germany.

The assessment of the sun protection factor is a well-known method, the SPF being the most decisive parameter for sunscreen labelling. Several methodological variables may influence the outcome of an SPF determination. The objective of this presentation is to give an overview of systematic research with a focus on: volunteers, light sources and product application. On the basis of a data pool combining the results of several SPF testing labs a weighting of the impact of the single parameters is possible. Concerning the UV sources to simulate sunlight, the influence of the spectral quality and of the total flux were investigated. As for the volunteers, we considered the phototype and the location of the test area on the back. Varying the product application procedure, we investigated the influence of application pressure and duration for spreading and different amounts of application. In result, we identify the UV light source as the most influential variable. The interindividual variation is the highest as such, but is partly ruled out by averaging over a sufficient number of subjects. The application procedure seems rather decisive for the variability of individual data but not so much for the height of the SPF.

IL009 New insights into the deleterious effects of UVA-radiation and development of novel photoprotective strategies
J. Krutmann; 1Institut für Umweltmedizinische Forschung, Düsseldorf, Germany.

We have analyzed the mechanisms by which UVA radiation induces gene expression in keratinocytes. UVA radiation-induced gene expression involved the formation of second messenger ceramide from cell membrane sphingomyelin, the subsequent release of mitochondria-derived cytochrome C and eventually the activation of transcription factor Activator Protein-2 (AP-2). Further studies identified signal-transducing microdomains (rafts) within the keratinocytes membrane as critical targets for UVA radiation. Thus, cholesterol and cholesterol-like molecules may serve a previously unrecognized role in photoprotection. In addition to direct effects on the expression of photoaging-related genes, UVA radiation causes photoaging skin through the generation of mitochondrial DNA mutations. We have shown in intraindividual studies that significantly high amounts of mt DNA mutations were present in photoaged skin, as compared to sunprotected skin. This is not merely an association, but mt DNA mutations are induced in human dermal fibroblasts by UVA radiation in vitro as well as in vivo in human skin. Moreover, mt DNA mutagenesis is associated with a profound disturbance of mt functions and an upregulation of matrixmetalloproteinase gene expression. These studies indicate that protection of mitochondria against UVA radiation-induced mutations and/or restoration of their function in photoaged human skin are novel and promising approaches to prevent and treat UVA-induced photoaging.

FC010 Photoprotection of some natural filters
E.C. Fernandes1; F. Kancan2; S. Rosany2; K. Boehm3; M. E. Hidalgo2; W. Quilhot4; C. Rustuc3; F. Thou2; H. T. Mayne2
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Lichens from high altitudes are subject to the effects of high UV radiation levels. Under this condition, they are stimulated to synthesize certain phenolic compounds, which presents a strong absorption in both UVA and UVB regions. In this work, we analyse the photoprotective performance of some phenolic compounds extracted from Chilean lichens. Most of the compounds tested in vitro demonstrated to have higher or similar filtering power than octylmethoxycinnamate, used as control. Ursinic acid—the most frequent metabolite in lichen species—resulted to be the best UBV filter, with an in vivo protection factor similar to Nivea sun Spray LSF5, used as reference. The protection factors suggest that these natural compounds may be useful in sunscreen preparations.

FC013 Assessment of the biological effects of solar UVA and irradiation and of the efficiency of photoprotection on reconstituted human epidermis
C. Masu1; A. Mazza1; P. Vicente2; 1Institut de Recherche Pierre Fabre, Castanet tolosan, France, 2Lab des MARCQ, UPS Toulouse, Toulouse, France.

The effects of solar UVA and irradiation with and without photoprotection have been assessed on reconstituted human epidermis (RHE). A multi-end-point analysis (MEA) was carried out, using histology, MTT assay and apoptotic response (sunburn cell counting [SBC] and p53 expression). The RHE was irradiated with solar doses ranging from 52.5 ml/cm2 to 420 ml/cm2, and with UVA doses ranging from 5 to 20 J/cm2. A broad-spectrum sunfilter formula was also tested at the higher doses (420 ml/cm2 and 20 J/cm2). In the absence of photoprotection, after solar irradiation at 420 ml/cm2 and 20 J/cm2, the number of SBC/cm2 increased to 41 & 22 respectively, and associated with a loss of viability. A response of p53 expression was observed, at first characterized by an overexpression. Subsequently a cleavage appeared, generating a 40 kDa fragment. When the RHE was protected and after irradiation, viability increased, and the number of SBC/cm2 decreased to 12 and 9 respectively. In addition, the p53 prototype disappeared, indicating that the photoprotection was effective. This work shows that RHE is a relevant model for use in the evaluation of the response to UV irradiation, as an alternative to human testing and to assess the photoprotective efficiency.

FC014 UVA Radiation Increases Melanoma Metastasis
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During sunbathing or tanning in solaria people are exposing their skin to large doses of UVA. We have examined whether UVA enhances metastatic potential of melanoma cells. C57BL/6 mouse-derived melanoma cell lines B16-F1 and B16-F10 and syngeneic MS-1 endothelial cells were used in experiments. First, we have examined in vitro effect of UVA on melanoma and melanoma-endothelium adhesion. Results suggest that UVA weakens melanoma-melanoma adhesion and enhances melanoma-endothelium binding. This effect, if occurring in vivo, might increase metastatic potential of melanoma. The suggested in vitro increase in metastatic potential of UVA-irradiated melanoma cells was confirmed in animal study. C57BL/6 mice were i.x. injected with B16-F1 melanoma cells and immediately after injection mice were exposed to UVA whereas the controls were not. Two weeks after injection, lung metastases were counted. In the controls we detected 2 metastases/10 animals, and in the experimental group we detected 27 metastases/10 animals. Histological evaluation confirmed that the nodules appearing in the lungs were melanoma derived metastases. Results of this study suggest that UVA might enhance metastatic potential of melanoma cells in mice. This observation further supports the notion of the urgent need for development of more effective UVA-absorbing sunscreens.

II015 Direct and photosensitized effects of the UV components of solar radiation on cellular DNA
J. Cadet1; S. Cordova2; J. Ravani1; E. Sage1; T. Dcou1; 1IER Grenoble, Grenoble, France, 2Institut Curie, Orsay, France.

Further insights into the photochemistry of cellular DNA were gained from the development of sensitive methods aimed at measuring base photoproducts. Thus, the twelve possible dimeric pyrimidine photoproducts including cis-syn cyclobutadiynegimines, pyrimidine-6-4-pyrimidone photoadducts and related valence Dewar isomers that are generated at TT, CC, CT and CC sites upon exposure of DNA to the direct effects of UVB radiation are now individually measured by high performance liquid chromatography associated with tandem electrospray ionization mass spectrometry (HPLC-ESI-MS). Interestingly, the distribution pattern of the main DNA photoprod-ucts is available for both monocytes and human fibroblast cells. The effects of less energetic UVA photons on cellular DNA are mostly explained in terms of photoproduction processes mediated by still unknown endogenous photo-sensitizers. Photodissociation reactions were found to lead to the preferential formation of cisp-oxo-8-oxo-7,8-dideoxyguanosine together with lower levels of oxidized pyrimidines and DNA strand breaks as inferred from HPLC-MS/MS and modified comet assay measurements. This was mostly rational-ized in terms of predominant involvement of singlet oxygen with a minor contribution of OH radical or related reactive species. However, the UVA pho-tosensitized formation of cyclobutadiyne and of relatively minor TC cyclobutadiyne appears to be more important quantitatively than oxidative damage so far identified.
we have analysed primary human skin fibroblasts and melanoma cells for the rect mechanism and to assess its relevance for the genotoxicity of sunlight, indirectly causing oxidative DNA modifications. To better characterize the indirect formation and repair of oxidative DNA damage induced by UV and visible light is one of blockage of transcription elongation and/or initiation. The mechanism of distorting lesions including UV induced photolesions. Three links with transcription and its interplay with transcription L. H. E. Mullenders, Department of Toxicogenetics, Leiden University, Leiden, Netherlands. The nucleotide excision repair (NER) system repairs a wide range of DNA helix distorting lesions including UV induced phototlesions. Three links with transcription exist: (i) active genes are repaired much faster than non-transcribed DNA, (ii) the basal transcription factor TFIIH is required for transcription initiation and NER and (iii) transcription is transiently repressed by DNA damage. The repression of transcription by DNA damage is likely to be a consequence of blockage of transcription elongation and/or initiation. The mechanism of UV induced transcription repression and repair was further examined by in vitro transcription and by a technique that allows irradiating only part of a cell nucleus. The results demonstrate that (i) repair and transcription factors are recruited to sites of damage, (ii) that inhibition of transcription after UV irradiation is at least partly due to repression of transcription initiation and (iii) that inhibition of transcription by trans is limited to short distance.

FC20 Immunochemical detection of pyrimidine dimers in nuclei of unstimulated lymphocytes S. A. Snapor1, F. R. de Gruijl2, L. Rozã¡3;
1Institute of Cytology, Russian Academy of Sciences, St Petersburg, Russian Federation, 2Department of Dermatology, Leiden University Medical Center, Leiden, Netherlands, 3Department of Nutritional Physiology, TNO Nutrition and Food Research, Zeist, Netherlands.

With the aim to study the processing of UV-induced DNA lesions in unstimulated human lymphocytes we used flow cytometry and fluorescent labelling of their partially denatured nuclei with the monoclonal antibody against cyclobutane pyrimidine dimers (aCPD-Ab). Following first hours of cultivation of UVC-irradiated cells we found an increase in aCPD-Ab specific fluorescence from cellular nuclei and a decrease in amount of aCPD-Ab-positive sites in isolated DNA from these cells. Both mentioned effects likely resulted from an excision-repair-related DNA modification, since they were prevented by a pre-treatment of the cells with a non-toxic dose of Novobiocin. While the partial loss of aCPD-Ab-binding sites from isolated DNA was obviously due to excision of a fraction of the dimers, the enhancement of aCPD-Ab-labelling of nuclei might be due to a formation of open structures at dipyrimidine-containing DNA fragments prior to excision. We suggest that formation of open structures predominated quantitatively over dual-incision and excision of these fragments and led to exposure of the pyrimidine dimers in nuclei to aCPD-Ab. Thus, unstimulated human lymphocytes appear to be capable of unwinding of DNA with pyrimidine dimers preceding the incision step. This unwinding appears to enable an initial increase in aCPD-Ab signal from nuclei.

FC21 DNA-Damage Assessment Applying Fluorescence Time-Resolved Measurements on Dye-DNA Complexes L. C. Scogg1, K. Focanou2, L. Mikelsaar1, C. Schneider3, A. Vinette4; 1University of Ottawa, Ottawa, ON, Canada, 2Radiation Protection Bureau - Health Canada, Ottawa, ON, Canada.

Several methods have been developed to measure DNA strand breaks in individual cells. Strand breaks in DNA result from direct scission of DNA by chemical attack; scission following the binding of intercalators, alkali-labile DNA adducts, endonuclease action, excision repair, etc. A quantitative determination of single-stranded DNA vs. double-stranded DNA is important to assess damage caused by external agents. We use the photophysical parameters of dye-DNA complexes to determine ratios of these two forms of DNA. The DNA-stain dyes are known to rotate freely in solution, but this non-radiative deactivation pathway is restricted for DNA-bound dyes. We have studied, using steady state and time resolved fluorescence, the complexes formed with different dyes and dsDNA and compared these results to those for dye-ssDNA complexes, where a less rotationally restricted dye should be present.

The fluorescence quantum yields and lifetimes for different dye-DNA complexes show that for intercalative binding dyes the dye-ssDNA complexes are more prone to deactivate nonradiatively than the dye-dsDNA ones. From the lifetimes for each of these complexes we derived a method for the evaluation of DNA damage. The method will be illustrated in relation to health and food authenticity issues.
IL024  
Tissue optical properties estimation for enhancing Laser Doppler Flowmetry  
T. Strömberg;  
Department of Biomedical Engineering, Linköping, Sweden.  
Microvascular blood flow as measured by a Laser Doppler Flowmetry (LDF) probe, is affected by the tissue optical properties (OP) and the probe geometry. We used a spatially resolved diffuse reflectance approach, with source detector distance up to 2 mm) to estimate OP. Theoretical modelling shows that for a homogenous distribution of moving scatters (blood cells), a path-length compensation algorithm can reduce the LDF perfusion estimation OP dependency. Measurements on a sophisticated flow phantom, however, showed that for flows at discrete depths, the OP dependency still remains.

IL025  
Laser induced photothermolysis of port wine stains present state of the art and possible improvements  
I. O. Svaastad;  
Norwegian University of Science and Technology, Trondheim, Norway.  
Laser induced photothermolysis is today the best modality for treatment of dermal vascular lesions, such as port wine stain (nevus flammeus). The optical wavelength is selected to give optimal absorption in blood, and the pulse duration is selected long enough to allow heat to diffuse into the vessel wall, but short enough to prevent diffusion into perivascular structures. The vessels can thereby be destroyed by thermal denaturation of the vessel wall by heating the wall up to about 70°C while leaving the perivascular tissues well below this threshold damage temperature. Most clinical procedures utilize a wavelength of 585 nm and a pulse duration of 4.5 ms. The acceptable radiant exposure, which must be limited to avoid unwanted damage to the epidermis, is usually selected in the range of 3–7 J/cm² dependent on the patient’s skin type. Selective cooling of the epidermis by oxygen spray cooling, which cools the epidermal/dermal junction down to 0°C, allows for an increase of the radiant exposure up to a factor of two. Dark purple port wine stains usually respond well to the first 3–5 treatments, and the skin shows improved blanching after successive treatments. However, in any cases where complete blanching is never reached, and the skin stabilizes at a pink-reddish color. Many pink-reddish port wine stains respond very often poorly even to the first treatments. The presentation will discuss the underlying physical principles of the treatment, and present possible techniques for improvement of the therapeutic outcome.

FC023  
UV-A/B-induced MMP expression in Squamous Cell Carcinoma  
M. C. Ramos1, H. Steinbrenner2, D. Stuhlmann3, H. Sies4, P. Brennere5;  
1Research Center for the Natural Sciences, Santo Tomas, Philippines, 2Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany.  
Exposure to ultraviolet radiation (UVR) has long been established to cause non-melanoma skin cancer, e.g. squamous cell carcinoma (SCC). Increased expression of matrix metalloproteinases (MMPs) by UVR was shown in numerous studies and supports the development of skin cancer. We were interested in the effect of UVR on induction of MMPs in the derived tumour cells. In our study, UV-A/B irradiation of an established cancer cell line, squamous cell carcinoma cell line (SCL-1), resulted in an induction and increased secretion of two members of the MMP-family, the interstitial collagenase (MMP-1) and stromelysin-2 (MMP-10), which could be shown by RT-PCR and ELISA. Up-regulation of MMP-10 steady-state mRNA level was already evident 1 hour after 30J/cm² UVA irradiation, while 30mJ/cm² UVB stimulated MMP-10 mRNA 8 hours after irradiation. The stimulation of MMP-1 occurs 4 hours after irradiation, while MMP-1 is stimulated 16 hours after UVB irradiation. In normal human dermal fibroblasts (NHDF), UVR-induced stimulation of MMPs peaked after 24 hours. The rapid stimulation of MMPs in SCL-1 cells correlates with an immediate UV-induced phosphorylation of extracellular regulated kinases (ERK-1/2) and p-38 stress kinase. By contrast, UVR irradiation of normal human epidermal keratinocytes (NHEK) and of an immortalized keratinocyte cell line (HaCat) did not up-regulate the MMPs.

FC026  
Optoacoustic investigations of human skin in the UV  
M. Meinhardt, R. Krebs, M. Bartels, A. Anders;  
Institute of Biophysics, Hannover, Germany.  
Optoacoustics provides depth resolved information about the optical properties of irradiated tissue. Our current tunable UV-laser system allows to gain this information for the whole UV-B range. In the near future, the construction of an improved system will extend the available wavelength range (ca. 285–400 nm) to the whole terrestrial UV range. Optoacoustics has been tested at the Institute of Biophysics in Hannover as a tool for the non-invasive investigation of the optical properties of human skin in the UV. Promising applications in vivo and in vitro will be presented. Our in vivo investigations include skin typing and signal variations due to different skin areas as well as the effects of substances, e.g. sunscreens, that are applied on the skin. Additionally, different skin types in vitro were examined by optoacoustics as well as other spectrometric methods and are compared to in vivo samples.

FC027  
Structural properties of GC polymers, DAPI complexed, studied by fluorescence correlation spectroscopy  
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1University of Catania, Catania, Italy, 2University of Illinois at Urbana-Champaign, Urbana, IL, United States.  
The binding of 4',6-diamidino-2-phenylindole (DAPI) to double-stranded GC polymers has been investigated using fluorescence techniques. We employed fluctuation correlation spectroscopy, which measures the diffusion coefficient of fluorescent particles, to demonstrate that the fluorescence was originating from relatively slowly diffusing entities. These entities display a very large heterogeneity of diffusing coefficients, indicating in our samples an extensive molecular aggregation. The fluorescence lifetime of the species was characterized using frequency domain fluorometry at different GC polymer/dye coverage. We observed a bright fluorescent component, at very low coverage, with a lifetime value of about 4 ns and the stoichiometry of binding was such that it can only arise from rare molecular structures, either unusual loops or large aggregates. The amount and characteristics of this component were different between the homo- and the alternating polymer, indicating that the difference in sequence of the polymers is responsible for the aggregates formation. At large coverage we observed a wide distribution of species with short lifetime values, in the range between 0.2 and 0.2 ns, likely arising either from intercalative and/or non-specific binding to the DNA molecules. We comment on the origin of the rare but bright fluorescent binding sites.
The role of carotenoids in protection of unsaturated lipids against photo-oxidation
M. Wang1, A. Pawlak2, M. Rozanowska3, T. Sarron4, J. Jagiellonian University, Krakow, Poland, 2Duke University, Duke, NC, United States.

Epidemiological data suggest that dietary carotenoids may decrease the incidence of many diseases including age-related macular degeneration (ARMD). It has been postulated that the protective effect of carotenoids is due to their ability to prevent peroxidation of polysaturated lipids. In particular, macular carotenoids could protect polysaturated lipids in the retina by absorbing potentially damaging blue light and neutralizing reactive oxygen intermediates generated by a variety of adverse processes. This paper will briefly review photochemical properties of macular pigments, particularly those relevant for their photoprotective action. In addition, we will discuss results of a new study aimed at analyzing the effect of exogenous carotenoids on oxidative injury induced by photoexcitation of rod outer segments (ROS) isolated from bovine retinas. Quenching of singlet oxygen was detected by time-resolved phosphorescence at 1270 nm. Progress of lipid peroxidation was monitored by ESR-oximetry, measurements of MDA-TBA, and HPLC chemical detection of cholesterol hydroperoxides. Our data show that pre-irradiation of bovine ROS with green light enhance their aerobic photoreactivity observed during photoexcitation at 355 or 406 nm. Zeazachin efficiently quenched singlet oxygen, generated by exogenous ROS photosensitizers, and inhibited the corresponding peroxidation of lipids. Supported by State Committee for Scientific Research (KBN 604003119)

ESP 2003 Abstracts
Methods: The corneal incident dose was 0–10 kJ/m². The intensity of light scattering sure groups: 100 SD rats received two UVR exposure separated by different exposure time groups: 100 SD rats were divided into 5 exposure time groups. The exposure time was 7.5–120 minutes. The corneal incident dose was 0–8 kJ/m².

Results: MTD increased with increasing age in the first 1/3 of life span and therefore easy to interpret and use. PPF values can be measured in-vivo but also a standard thickness of stratum corneum. The PPF values are linear and therefore easy to interpret and use. PPF values do not only include the UVR protection from pigmentation alone but also a standard thickness of stratum corneum. The PPF values are linear and therefore easy to interpret and use. PPF values can be measured in-vivo but also a standard thickness of stratum corneum. The PPF values are linear and therefore easy to interpret and use. PPF values can be measured in-vivo but also a standard thickness of stratum corneum.

Conclusion: Young rats are more sensitive to UVR than old rats. The factors of exposure time and repeated exposure with different time intervals have no influence on MTD for the same UVR dose for UVR induced cataract.

Exposure of the dark-adapted retina to intense light leads to accumulation of high concentrations of toxic all-trans retinal (RAL) in photoreceptor outer segments, substantial amounts of which are complexed with phosphatidylethanolamine (PE). The aim of this study was to determine the effects of PE on photosensitising properties of RAL and its (photo)toxicity to the retinal pigment epithelium cells. RAL was incorporated into liposomes made of phosphatidylcholine (R/PC) or a mixture of PC and PE (65:35 mol/mol; R/PE). The factors of age, exposure time and repeated exposure with different time intervals have no influence on MTD for the same UVR dose for UVR induced cataract.

Studies have shown a good relation between the PPF values and Minimal Erythema Doses (MED) found by Photo tests on both sun unexposed buttock skin (r² = 0.78) and sun exposed upper back (r² = 0.68). We have found a relation between PPF values on different body locations and Skin Type (346 Subjects). The strongest relation was not found on constitutive buttck skin, but on the upper back/shoulder: Skin Type I: PPF 5.6, II: PPF 6.7, III: PPF 7.4 and IV: PPF 8.3. PPF value is more sensitive, fast and easily measured, and could therefore replace both self-reported Skin Type and Phototest in establishing UVR sensitivity in people with normal skin.

The extent to which visible “melanin” may be used to predict skin reactivity to ultraviolet radiation

ESP 2003 Abstracts
UILO43 Fluence rate determines the inflammatory response in photodynamic therapy of tumors
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Roswell Park Cancer Institute, Buffalo, NY, United States.
The fluence rate at which photosensitizer-activating light is delivered can greatly influence the oxygenation status of a tumor during treatment, as well as treatment outcome. Here we present evidence that fluence rate can also determine the local inflammatory response. PDT using the photosensitizer HPPH was administered to mice carrying the Colo 26 carcinoma, employing fluence rates of 14 or 112 mW/cm² for a fluence of 48 or 128 J/cm². The low fluence rate regime maintained tumor oxygenation during light delivery, the high fluence rate regime depleted oxygen. Levels of cytokines, inflammatory host cells and apoptosis were assessed. All treatment conditions elicited an inflammatory response, the extent of which varied markedly. Low fluence rate at low fluence produced an extremely strong inflammatory response, while low fluence rate at high fluence elicited a minimal response. This was in contrast to the therapeutic outcome which was the lowest under low fluence rate/high fluence conditions. High fluence rate treatments elicited intermediate levels of local inflammation and poor tumor responses. These data suggest that a) highly effective PDT can limit the local inflammatory response and b) that the inflammatory response is not a general controlling factor in the therapeutic outcome of PDT.

UILO44 Activators of the alternative complement pathway are highly efficient adjuvants to photodynamic therapy for cancer treatment
M. Korbelik1, J. Sun1, I. Cecic1, P. Cooper2;
1British Columbia Cancer Agency, Vancouver, B.C, BC, Canada, 2Autoimmunity Research Unit, The Canberra Hospital, Vancouver, B.C, BC, Canada.
Treatment of solid tumors by photodynamic therapy (PDT) triggers a strong host reaction resulting in the engagement of effector mechanisms coordinated to inflammatory and immune responses. A major element of the elicited innate immune response is the activated complement system, as it drives the propagation of both inflammatory and immune processes involved in the eradication of PDT-treated tumors. Findings from our studies based on mouse tumor models that characterize the mechanism of complement activation following PDT will be shown. This will be followed by the report of our investigations exploring the perspectives of a further amplification of complement activity with an aim to increase the cure rates of PDT-treated tumors. It will be shown that the activators of the alternative complement pathway, such as zymosan and gamma-inulin, injected directly into the lesions immediately after PDT, produce a dramatic improvement in the therapeutic outcome, even of highly resistant poorly immunogenic solid cancers.

FC045 Use of ESR and HET-CAM-Assay in investigation of phototoxicity
B. Algermissen, B. Jamil, A. Krink, D. Mangoldt, C. M. Philipp, H. Berlin;
Riklimkum Neukoelln, Berlin, Germany.
In the future PDT after systemic application of a photosensitizer will become more important. One of the main side effects is the acute and prolonged photosensibilisation and resulting phototoxic side effects. The aim was to use ESR and HET-CAM-assay to analyse different lamps for their ability to induce singulet oxygen and phototoxic effect. Different lamps (fluorescent light, sodium light) were tested for generation of singulet oxygen and phototoxic reaction. A solution of hematoporphyrin (1μg/ml), OH-tetramethylpyyridine (10 μM) and HET-CAM assay were exposed to the light sources and the resulting product TEMPO analysed using electron spin resonance technology (MiniScope, Magnetech, Berlin). Phototoxic reactions were analysed using the HET-CAM assay. HPPH were applied on the CAM and exposed to light sources. Alterations of the CAM were analysed. Excellent correlations were found between the data achieved with the in vitro ESR system and the in vivo HET-CAM-assay. Therefore the production of singulet oxygen and the generation of damages or thrombosis of the CAM vessels seems to depend on the rate of singlet oxygen generation. Interestingly, compared to fluorescent light the sodium lamp produced lower generation of singulet oxygen and also lower distractions of the CAM-vessels at the same light intensities.
FC046 Suppression of contact hypersensitivity in mice by products of protoporphyrin IX photosoxidation
A. V. Potapenko1, A. A. Krygova1, G. V. Mansurova1, L. A. Kozir1, V. Y. Pavlov1, I. O. Konstantinova1, G. V. Ponomarev2
1Russian State Medical University, Moscow, Russian Federation,
2Institute of Biomedical Chemistry, Moscow, Russian Federation.

Photodynamic therapy (PDT) is known to be accompanied by immuno-suppression. Photophysical mechanisms underlying this effect are not clear. Here we explored immuno-suppressive activity of photosensitization products of protoporphyrin IX-dimethyl ester (PPIX) by employing murine model of contact hypersensitivity reaction (CHS) to 2,4-dinitrofluorobenzene. Photosensitization (365 nm) of PPIX solution yielded two major chlorin-type products, namely, dimethyl ester photoprotochlorophyll (I) and iso-photoporphyrin (II), and several minor unidentified compounds. Intra-vaneous injection of crude mixture of photosensitizers resulted in fluorescence-dependent suppression of the CHS. Dose dependent suppression of the CHS was also induced by i/v injection of purified I or II. About 50% suppression was achieved at PPIX preirradiation fluence yielding totally about 0.1 μM of I and II. The same extent of suppression was induced by injection of purified 0.1 μM solutions of I or II. These observations strongly indicate that photosensitization products of photosensitizers can contribute to immuno-suppressive effects of PDT.

FC047 Photosensitizers and photoproducts identification by Mass Spectrometry in whole cells
N. Lourette1, B. Maun1, J. Muller1, L. Bezdetnaya2, J. Guillemin1,
1Université de Metz, Metz, France, 2Unité de recherche en Thérapie Photodynamique, CNR-CNRS UPRS-J 7039, Centre Alexis Vautrin, Vandoeuvre les Nancy, France.

In photodynamic therapy (PDT), a photosensitizer, 5,10,15,20-tetrakis(m-dioxo-5,7-dimethylphenyl) chlorin (m-THPC, temoporfin), administered to patients generates cytotoxic species after light irradiation. The challenge of our study consists in doing the identification of the cytotoxic species directly on the cells, by Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF/MS) after photosensitizer impregnation and laser irradiation. In a first step, we analysed HT29 human colon adenocarcinoma cells by MALDI-TOF/MS. Analyses by mass spectrometry allowed to detect a proteinic fingerprinting from 0 Da to 20,000 Da, different from the one of the culture medium. Then, cells were incubated with m-THPC with three different concentrations (5, 10 and 20 μg/mL) during 3h30. We succeeded in detecting the m-THPC in a entire cell. In order to observe photoproducts, cells were exposed to 652 nm light from laser diode with four fluences: 1, 5, 10 and 20 J/cm². This experiment showed that the most important the signal fluence, the weaker the m-THPC signal. We were able to observe m-THPC photobleaching and the results concerning photoproducts will be developed during this communication.

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A. V. Potapenko1, A. A. Krygova1, G. V. Mansurova1, L. A. Kozir1, V. Y. Pavlov1, I. O. Konstantinova1, G. V. Ponomarev2
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IL051 Methods of objective assessment of non-ablative skin-laser interactions
C. Edwards;
Royal Gwent Hospital, Newport, United Kingdom.

While some skin-laser interactions are dramatic and immediate, many are progressive, with small incremental changes which may take some days or weeks to fully occur. Methods of assessment of such changes have, in many cases, been confined to clinical observations. Results such as “65% of patients reported at least 75% improvement” can be very useful in indicating whether a treatment is worthy of implementation or of further study. However, such statements do not give ‘hard’, or quantitative, data. This makes comparing studies or techniques very difficult, and does not allow precise judgment of subtle differences between instrumental settings or between different instruments. Following the progressive course of treatments requiring multiple laser sessions is also not easy using descriptive methods. Even good clinical photographs are, without measurements, at best a guide to progress. This paper will review simple and well-established methods of assessment which can be used to measure the effects of laser treatments on skin. Such measurements include area, colour, blood flow, skin thickness, volume, skin roughness. Examples of the use of quantitative techniques include the measurement of volume of keloid scars, the measurement of colour of port wine stains, and the measurement of hair removal.

IL052 Semiconductor Light Sources for photon therapy and photosensing applications
G. Jones;
Enfis Ltd, Swansea, United Kingdom.

Font face-rial semiconductor light sources are continuing to emerge as key enabling devices for medical applications. In particular, semiconductor laser diodes (LD) and light emitting diodes (LED) are becoming widespread in both the therapeutic and diagnostic fields. The purpose of this paper is to present a general overview of the characteristics of semiconductor LD and LED sources. Furthermore these characteristics are compared with the requirements for certain photobiological applications explaining how the attributes of these types of sources lead to their use in photobiology. The paper will focus on the technology aspects of the devices such as the spatial, spectral and temporal characteristics and how these are used within photobiology. For example, in the field of phototherapy such devices are now being used in dermatology for treatment of acne or psoriasis, for the removal of unwanted hair and the treatment of port wine stains. In conjunction with drugs during pdt both led and ld devices provide highly efficient activation sources. In photosensing such applications the determination of skin type or colour or bacteriological activity provides essential diagnostic support as remote imaging and diagnosis becomes more pervasive in society. Applications once reserved for the laboratory are now being provided as commercial systems for use in the clinic and even the home as the lower cost and smaller semiconductor sources take over from the traditional lamp and laser devices.

IL049 Selective photothermolysis of blood vessels using indocyanine green and laser irradiation
P. Babilar, V. Schacht, R. Engl, H. Stockmeier, W. Baemmler, R. Szeimies, C. Abels;
University Hospita Regensburg, Regensburg, Germany.

To destroy blood vessels selectively lasers with a wavelength of 585 nm are used matching the absorption of hemoglobin. Since light penetration into tissue at this wavelength is limited, indocyanine green (ICG) was used absorbing at 805 nm. The skinfold chamber model in hamsters was used for monitoring the vascular effects following ICG (0, 2 or 4 mg/kg b.w.; ICG-Pulsion, Munich, Germany) and diode laser irradiation (λ = 805 nm; pulse duration: 3, 10 or 30 ms). Diameters of vessels marked with FITC-dextran were measured using intravital fluorescence microscopy prior to and up to 24 h following irradiation. Irradiation with a pulse duration of 30 ms without ICG did reduce the number of perfused vessels temporarily by 4%. Irradiation (10 ms) using 2 mg/kg b.w. ICG reduced the number of perfused vessels 1 h after irradiation and recovered at 24 h as well. Using 4 mg/kg b.w. ICG and a 30 ms pulse duration reduced the number of perfused vessels by 53% at a diameter of 7–8 μm permanently. The selective vascular damage was confirmed by histology. This study shows the selective photothermolysis of blood vessels using intravenous injection of ICG and irradiation with a pulsed diode laser.

FC053 Pulsed Dye Laser treatment of sebaceous gland hyperplasia
M. M. Soliman;
National institute of laser enhanced sciences, Guiza, Egypt.

Sebaceous gland hyperplasia may be treated by cryotherapy, electrical cautery, chemical cautery, surgical excision or CO2 laser vaporization. All these modalities carry the risk of scarring or pigmented changes. The aim of this study is to assess the efficacy of pulsed dye laser for the treatment of seba- ceous gland hyperplasia. Twenty patients with multiple sebaceous gland hyperplasia were treated by pulsed dye laser (candela corp, USA) [585nm,450usec] using fluence range from 6.5–8–J/cm, and spot size 5 mm & 7 mm with overlapped pulses. Number of sessions is 2–3 session with 4 weeks interval. All lesions responded immediately to the first treatment and completely gone after the third session. No scarring or pigmented changes were seen after 12 months follow up. Pulsed dye laser may be an alternative treatment for sebaceous gland hyperplasia with minimal risk of scarring or other side effects.
FC054
Histopathological study of Xanthelasma Palpebarum after Pulsed Dye Laser Treatment
M. M. Saliman,
National Institute of laser enhanced sciences, Guiza, Egypt.

Introduction:
Xanthelasma Palpebarum is the most common type of xanthomas. Treat-
ment modalities are multiple, they could be surgically removed, chemically
cauterized, electrically cauterized, or by the use of CO2, or Pulsed Dye Laser.
Patients and Methods:
30 patient were treated using Pulsed Dye Laser [585nm,450us] with en-
ergy density ranged from 6.5 to 7.5 J/cm, and spot size 5 mm using overlapping
pulses, and repeated sessions up to 4. Histopathological examination, pre and
post laser was performed.
Results:
Post Laser histopathological examination revealed the disappearance of xan-
thoma cells completely after an average of 3 Laser sessions, and this corre-
lates with the clinical results.
Conclusion:
Histopathological results confirmed the excellent curative and cosmetic
correlates with the clinical results.

IL056
Inner retinal photoreceptors in mammals
R. J. Lucas1, R. G. Foster1, R. H. Douglas1, M. W. Hinks1, S. Thompson3,
1Imperial College London, London, United Kingdom, 2City University, London,
3Johns Hopkins Medical Institute, Baltimore, MD, United States.

In mammals a variety of non-image forming light responses including circad-
ian phototentrainment and pupillary constriction are capable of employing
ocular photoreceptors other than the classical rods and cones. Recent evi-
dence suggests that these photoreceptors comprise a subset of retinal gan-
glion cells that express the putative photopigment melanopsin. These cells
are intrinsically photosensitive and project to brain areas that are responsible
for non-image forming visual responses. We present evidence that melanopsin
is critical to the photosensitivity of these retinal ganglion cells
and that these inner retinal photoreceptors act in concert with the rod/cone
systems to drive a variety of light responses in mammals.

IL075
Divergent mechanisms for the tuning of shortwave sensitive visual
pigments in vertebrates
D. M. Hsu
Institute of Ophthalmology, London, United Kingdom.

The shortwave-sensitive (SWS) visual pigments of vertebrate cone photore-
ceptors are divided into two classes on the basis of molecular identity, SWS1
and SWS2. Only the SWS2 class are present in mammals. The SWS1 pigments
can be further subdivided into violet-sensitive (VS), with λmax values general-
ly between 400 and 430 nm, and ultraviolet-sensitive (UVS), with λmax values
<380 nm. Phylogenetic evidence indicates that the ancestral SWS1 pigment
was UVS and that this pigment has remained UVS in all fish and reptilian
species so far examined. In birds however, it has evolved secondarily from a VS
pigment via a unique Ser90Cys substitution. The shift from UVS to VS has
resulted in increased sensitivity and response amplitude of mutant respons-
ees. Interestingly, pigment is found to accumulate on long term dark adapta-
tion and visual function improves. The characterization of this pigment will be
discussed. Administration of 11-cis retinal to these animals partially restores the
rod visual pigment and that this pigment is functional. In addition, we find
that if the 11-cis retinal is administered to young animals c3 weeks), the
cones can be partially preserved and cone function is likewise maintained.

IL059
The Molecular Mechanisms of Congenital Night Blindness
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Three rhodopsin mutations, Q90D, T94L and A292E, have been found to cause
congenital night blindness (CNB) in humans. Two models have been proposed
to account for how the mutation Q90D causes CNB: one involves constitutive
activity of the apoprotein opsin; the other suggests that an increased rate of
tissue isomerization of the visual pigment rhodopsin is responsible.
We have designed combined molecular genetic and electrophysiological experi-
ments to determine which mechanism is correct. Transgenic Xenopus laevis
were generated in which the Q90D, T94L, and A292E mutations of rhodopsin
were expressed in the major rod photoreceptor cells. Electrophysiological
measurements of sensitivity and response kinetics were made in darkness
before and after treatment with 11-cis retinal. Mutant responses displayed
accelerated dim-flash response kinetics and desensitization. Administration of
exogenous 11-cis retinal (50 μM - 250 μM in Ringer/ETOH (0.1%) for 5 min)
resulted in increased sensitivity and response amplitude of mutant respons-
es and slowing of dim flash response kinetics to levels similar to dark-adapted
wild-type rods. Our results suggest a model in which abnormally high con-
stitutive opsin activity is the basis for the disease and inconsistent with the
thermal isomerization model.

FC061
Photodegradation dynamics and rates of bacteriorhodopsin
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The rates of photodegradation of bacteriorhodopsin (br) were measured over the
wavelength range of 450 to 650 nm using a 10 hz pulsed laser light
source operated between 4 and 15 mW/cm. The action spectra show signifi-
cant photodegradation rates between 480 and 540nm, which corresponds to the
strong visible absorption band of bacteriorhodopsin. The kinetic data
implies a first order reaction mechanism leading to photodegraded br, but
it also implies that the br is simultaneously driven down an alternate reac-
tion pathway to form the previously reported "blue state". The reaction to the
"blue state" is irreversible, and hence, as the br concentration is depleted due
to the photodegradation reaction, the equilibrium shift drives the "blue state"
back to the starting material, br.

IL062
Are Dietary Carotenoids Beneficial? Reactions of Dietary Carotenoids with
Oxygen Radicals and Singlet Oxygen
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Carotenoids play diverse roles in biology and medicine. Both the quenching
of singlet oxygen (energy transfer) and interaction with oxaradicals (electron
transfer, H-atom transfer and addition reactions) are key processes in under-
standing many of these roles. Much previous work in “simple”solvents is
reviewed and new results in cell membrane models are presented. The
possible consequences of using carotenoids as dietary supplements are dis-
cussed.
Stratospheric ozone and climate change

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Systematic temperature measurements of the lower stratosphere obtained from satellite microwave sensors with global coverage show a cooling trend of about 1.5°C since 1979. At the same time the temperature has risen in the lower troposphere reaching a maximum of some 0.4°C close to the surface. The stratospheric cooling is larger at higher latitudes, in particular at the Southern Hemisphere where the cooling trend amounts to several degrees Celsius. The surface warming, on the other hand, is largest in the Arctic and sub-Arctic region with almost no warming in the polar region of the Southern Hemisphere. Modelling studies done over the last few years show that the evolution of temperature of the atmosphere and of the Earth climate in general is the result of many different causes including dynamical processes between the oceans and the atmosphere, volcanic eruptions and changes in the composition of the atmosphere such as increasing anthropogenic greenhouse gases and emission of long-lived trace gases capable of stratospheric ozone destruction. Studies performed in cooperation with the Max Planck Institute of Chemistry in Mainz demonstrate that ozone loss in the stratosphere is the main cause of stratospheric cooling. It is highly unlikely that natural processes are the cause. The ozone reduction also contributes to reducing the warming in the upper troposphere, thus bringing modelling results in better agreement with observations. Recent measurements indicate that stratospheric cooling is levelling off as a consequence of stabilising the ozone concentration due to the successful Montreal agreement (and following agreements) to reduce the emission of ozone-depleting substances. However, many problems still remain. A particularly important question is related to possible changes in the atmospheric circulation due to greenhouse warming which could give rise to positive feedback effects, again worsening the negative effect of a lower concentration of stratospheric ozone.

Ozone/uv relations under changing future atmospheric composition

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Increases in the chlorine loading in the stratosphere has led to significant depletion at high northern and southern latitudes over the last decades, resulting in large increases in the penetration of harmful ultraviolet radiation to the earth’s surface. Reduction in uv radiation due to recovery of the stratospheric ozone layer, from reduced emissions of man made chlorine compounds, will be strongly affected by the long-term changes in compounds like ch4 and n2o. n2o, a greenhouse gas with a strong long-term effect, and ch4, which has a shorter atmospheric lifetime, both contribute to the changes in the stratospheric and lower tropospheric ozone concentrations. In the future, the stratospheric ozone concentration is expected to recover, and the main uncertainty is related to the timing of this recovery.

The UV algorithm has been successfully tested against ground based measurements, together with a range of data products for a variety of applications. A data product developed at UMIST provides daily and monthly UV data for any location on the globe, based on total solar radiation,inline turbidity, column ozone and precipitable water vapour. The data required for the UV algorithm are retrieved or calculated within SoDa, or input by the user. The quality of the input data determines the quality of the output and best results are obtained when measured data is available within SoDa rather than climatological or derived values. The UV algorithm has been successfully tested against ground based measurements from both the UK and Thailand.

The influence of Mini-ozoneholes to the biologically effective ultraviolet radiation over Central Europe

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We have investigated the impact of MOHs to the biologically effective UV radiation using TOC measurements made from space and from the ground. For this study we introduced three different photobiological effects by applying the action spectrum of the erythema (EY), that of the generalised plant damage (GPD) and that of DNA damage (DNAD). Two extreme MOHs occurred over Central Europe within the last 5 years, whereas TOC falls below 200DU. The first was recognised in January 1998, the second in December 1999. During these MOHs a decrease in order of 50% could be observed with a few days. The increase of the biologically effective UV radiation however was much higher. The highest increase was observed in the GPD irradiance (E-GPD). During the MOH of 1998 E-GPD increases from 1.5 mW/m² to 19 mW/m², during the MOH of 1999 from 3 W/m² to 30 W/m². For DNAD the effect is less dramatically. DNAD was starting from approximately 0.5 mW/m² and reached a level of 3.5 mW/m² during MOH 1998, respectively from 0.9 mW/m² to 5.4 mW/m² during MOH 1999. Although the lowest increase was found for the E-EY, the corresponding increase was derived being a factor 3 to 4 for both MOHs.
IL074

Heat shock proteins in photoaging and photocarcinogenesis
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All organisms respond to sudden environmental changes with the increased expression of heat shock proteins (hsp). Hsp mediate a transient state of increased resistance to further stress challenge. The high evolutionary conservation of this response and of the involved genes suggests their importance for survival under hostile environmental conditions. Skin exposure to ultraviolet radiation is such a condition that can lead to immediate and chronic photodamage and hsp might play a role in their prevention and/or repair. It has been shown that human epidermis contains high levels of hsp72 without prior stress exposure and further stress challenge leads to upregulation. Induction of hsp by hyperthermia or overexpression is able to inhibit UV-induced cell death. Current studies investigate the mechanisms of this anti-apoptotic effect and of the interactions of hsp with DNA damage and repair. With age the general amount and the inducibility of hsp is reduced in most organs and tissues. In skin this might contribute to a decreased defense against photodamage. Based on this experimental data it is been proposed that pharmacologic interventions to restore the heat shock response in aging skin might be useful as a novel concept for the prevention of photoaging.

IL075

Photoaging of the Human Eye
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Although the human eye is constantly subjected to ambient radiation, there is little damage to the eye from light until middle to old age. The reason is that there are yellow chromatophores in the human lens (3-hydroxykynurenine) that absorb light but are photochemically inactive. They serve to protect the retina by filtering UV light and preventing it from reaching and damaging the retina. After middle age an enzyme (kynurenine amino transferase) is produced in increasing amounts which converts the protective 3-OH kynurenine and its glucoside into phototoxic chromophores, xanthurenic acid and its glucoside which induce age-related cataract formation. Because of the filtering characteristics of the lens, only visible light (above 400 nm) reaches the adult retina. Normally, this visible light does not damage the retina. However, with age, the human retina gradually accumulates a fluorescent material, lipofuscin that contains an as yet undetermined phototoxic chromophore, which induces photoreceptor degeneration. With middle age, these phototoxic chromophores are forming, the protective antioxidants begin to deplete in the eye. It is for this reason that exposure to sunlight or very intense light either causes or exacerbates blindness in old age.

IL076

Objective assessment of photoaging effects using high-frequency ultrasound in PUVA-treated psoriasis patients
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Background: Skin aging can be differentiated into intrinsic (chronological) aging, and photoaging due to chronic sun exposure. Photoaging is the superimposition of photodamage on the aging process.

Objectives: The aim of the study was to investigate possible differences between the skin of photochemotherapy (PUVA)-treated patients and of untreated normal subjects using a high-frequency ultrasound system.

Methods: A total of 124 volunteers (aged 21–88 years, median 52 years, 62 female, 62 male), 62 psoriasis patients who had received PUVA therapy and 62 healthy controls, were investigated. Skin thickness and a subepidermal low-echogenic band (SLEB), a parameter for photodamage, were measured in 12 different areas. Results: Female skin is thinner than male skin. The skin of PUVA patients were more markedly decreased than those of the controls for the older patients. There was a clear dependence of the occurrence of SLEB on PUVA therapy in psoriasis patients.

Conclusions: Long-term PUVA treatment in psoriasis patients accelerates thinning of the skin in comparison to age-matched controls. The results show that ultrasoundography is a sensitive method to investigate the effects of PUVA-induced skin aging.
IL079
A Genomic Analysis of the Shade Avoidance Response
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In response to the proximity of neighbouring vegetation, many species of plants dramatically alter their architecture in order to prevent shading by competitors. Light reflected from neighbouring vegetation is specifically depleted in red light following absorbance by chlorophyll. This change in light quality is detected by the red and far-red absorbing phototropes and induces a classical “shade avoidance response” – increased in elongation growth, decreased branching and accelerated flowering. We know very little about the changes in gene expression involved in this response. Using Affymetrix microarrays we have characterised genes differentially expressed under different RFR ratios in Arabidopsis. We compared gene expression in phototropin/phototropin-like mutants vs wild-type seedlings at both early and late time-points. We have identified numerous shade-regulated genes showing a variety of patterns of regulation by the different phototropes. This list includes many putative novel transcription factors as well as elements previously identified in plant hormone signalling pathways.

IL080
The bacterial counterparts of higher plants phototropins
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The photosensory receptors phototropins (phot) are light-driven kinases that regulate a variety of blue-light responses in plants, such as phototropism, stomatal opening and chloroplast relocation (Briggs and Christie, 2002). Through a combined approach that couples genome digging and recombinant protein expression, similar proteins (phot-related) have been discovered in bacteria (Losi et al., 2002). In phot-related bacterial proteins, the flavin binding phototropin LOV domain (from Light Oxygen and Voltage) is coupled to different downstream kinase modules. We have identified phototropin-related kinases, response regulators. The photochemistry, photophysics and thermodynamics of these kind of proteins from Bacillus subtilis (YtvA) and Caulobacter crescentus (sensory tors) identifies fit with previous studies showing that the furocoumarin photolytic at the furan ring by singlet oxygen produces active photoproducts. The result compound, 4'-aminomethyl-4,5,8-trimethoxyfurocoumarin, has recently been used to prevent Graft versus Host Disease (GVHD) associated with stem cell transplantation (SCT) (4) and for the creation of a vaccine for Epstein-Barr Virus (EBV).

IL082
Photochemical Crosslinking Of Microbial Genomes As An Approach To Derive Vaccine Platforms That Combine Immunopotency With Safety
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We have developed a novel approach to vaccine development that combines recombinant gene-based vaccines and for organism-based vaccines against microbial pathogens. This strategy utilizes whole microbes that are non-viable, yet retain their ability to induce robust immune responses. As an example, we have engineered Listeria, an intracellular Gram-positive bacterium, to be exquisitely sensitive to inactivation by psoralens. Listeria unable to repair psoralen-mediated DNA damage were created by deleting the ultraviolet light resistance (uvr) AB gene. Listeria uvrAB were four logs more sensitive to psoralen/UV-A light inactivation. However, since they were metabolically active, inactivated Listeria uvrAB bacteria retained their ability to infect APC, escape from the phagolysosome, and induced robust functional CD8+ antigen-specific T-cells and therapeutic anti-tumor efficacy in immunized mice. We believe that this technology can be utilized as a general approach to generate safe vaccines that are genetically inactivated, yet retain the capacity to be taken up by antigen presenting cells, express their genomic repertoire, and, importantly, program the presentation of antigens relevant to cancer or infectious disease. This technology can be applied to recombinant bacterial-based vaccines and to whole pathogenic organisms, where sub-unit vaccines are poorly immunogenic, or for which the correlates of protection are unknown.

IL083
The pivotal role of mitochondria in psoralen-induced apoptosis
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The combination of psoralen and UVA irradiation, commonly referred to as PUVA, represents a useful therapeutic approach based on the ability of this treatment to induce cell death. Although apoptosis has been reported to result from PUVA treatment, scarce information is available concerning the underlying mechanism. The mechanism of cell death was investigated in Jurkat cells exposed to PUVA. Apoptosis was by far prevailing over necrosis and involved mitochondrial dysfunction. The collapse of mitochondrial membrane potential appears to becaused by the opening of the mitochondrial permeability transition pore since its inhibitor, cyclosporin A, prevented mitochondrial dysfunction and largely attenuated apoptosis. Interestingly, apoptosis also occurred in cells treated with the photoproducts (POP) generated by irradiating psoralen in vitro in the presence of oxygen. The analysis of the apoptogenic potency of the HPLC-separatet POP fractions and their structur-
The role of photoactivated psoralens in the cell cycle

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Although many aspects of PUVA therapy have been studied extensively, the molecular mechanism by which it kills cells has not been elucidated. In this study, we have investigated the biological activity of four psoralen derivatives: 5-methoxypsoralen (5-MOP), 8-methoxypsoralen (8-MOP), Angelicin (ANG) and 4,6,4'-trimethylecyclomellinene (TMA) in two human tumor cell lines such as HL-60 a promielocytic leukaemia and HT-1080, a fibrosarcoma. The results proved HOFLQ leads to a specific damage, different from that of typical furocoumarins.

FCO87
Psoralen photoproducts with possible apoptotic activity
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PUVA has been shown to have apoptotic effects on several cells. Irradiation of psoralen in aerated water-methanol solution yielded a complex mixture of photoproducts arising from both oxic and anoxic mechanisms. When this mixture was added to Jurkat cells, it also induced apoptosis, although at a lesser extent than PUVA (at about the same drug concentration and light dose). This behaviour suggests that cell damage should come partly from direct photochemical injury and partly from some stable photoproduct. The mixture was roughly divided into 6 fractions by HPLC and the fraction tested directly in the experiments (incl. TPA). The regimens were about equally effective in inducing papillomas. Hence, the intermittent overexposure to UVB appeared to be selective for the promotion of nevi.

IL090
Tumor development in hairless Xpo Ink4a/- mice under various neonatal and adult UV exposure regimens
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Defects in the Ink4a tumor suppressor locus are associated with familial melanomas in humans, and defects in nucleotide excision repair (eg Xpa) are associated with an increased risk of sporadic melanoma. To introduce a bias toward melanocytic tumors we used hairless Xpo Ink4a knockout mice, and exposed them to various carcinogenic regimens: either DMBA (50 ul 0.2% in acetone) or 4 MED UVB (TL12 lamps) neonatally, followed in adulthood by either 2 times weekly exposure to TPA or regular UVB exposure: 0.6 MED/day, 4 MED/week or 6 MED fortnight. Nevi, papilomas, carcinomas and other types of tumors were observed, but no malignant pigmented tumors. Neonatal DMBA proved more effective in inducing nevi than UV exposure, and 6 MED every fortnight proved more effective than the other adult treatments (incl TPA). The regimens were about equally effective in inducing papilomas. Hence, the intermittent overexposure to UVB appeared to be selective in enhancing melanocytic hyperplasia, which is reminiscent of the finding that episodes of severe sunburns are associated with increased melanoma risk in humans. The observations thus far indicate that an Ink4a deficiency does not enhance the formation of nevi, nor is it sufficient for the malignant progression of nevi.

IL093
Role of p53 and Fas ligand in PUVA-induced apoptosis and carcinogenesis
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A combination of Psoralen and UVA radiation (PUVA) is widely used in the treatment of psoriasis and other skin diseases. Even though PUVA therapy is highly effective in eliminating hyperproliferative keratinocytes in the epidermis, its mechanism of action has not been fully elucidated. In this study, we used JB6 mouse epidermal cells, p53-/-, and Fas ligand-deficient (gld) mice to investigate the molecular mechanisms by which PUVA induces cell death. Treatment of JB6 cells with 10 µg/ml of 8-methoxypsoralen followed by 20 kJ/m² UVA irradiation resulted in cell death by apoptosis. In addition, PUVA treatment resulted in p53 stabilization, phosphorylation at serines 15 and 389, and nuclear localization as well as induction of p21Waf/Cip1 and caspase 3 activity in vivo studies revealed that PUVA treatment induces significantly less apoptosis in the epidermis of p53-/- mice compared to p53+/- mice. Furthermore, gld mice were completely resistant to PUVA-induced apoptosis compared to wild-type mice. These results indicate that acute PUVA treatment induces apoptosis in mouse epidermal cells in vitro and in vivo and that p53 and Fas/Fas ligand interactions are required for this process. However, chronic PUVA treatment results in dysregulation of apoptosis and induction of p53 mutations leading to development of skin cancer.
FC94 The Contribution of calpains in the downregulation of MDM2 and p53 pro
telosyis in reconstructed human epidermis in response to solar irradiation
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p53 is critical in preventing the genome from incorporating damaged DNA, particularly in the skin. It is also known that a relevant stress that activates p53 function is UV light. The cellular regulation of p53 stability is important in the maintenance of cell integrity, but its mechanism is unclear. Upon UV irradiation, p53 protein accumulates in vitro and in vivo human skin cells. This transitory stability of p53 requires a decrease in the activity of the ubiquitin ligase MDM2. In this work, solar light stimulation of reconstructed human epidermis at first caused an accumulation of p53 protein, along with a decrease in the level of expression of MDM2. Then, 24 hours after irradiation, a specific cleavage of p53 resulted in the formation of a 40 kDa fragment. In the presence of calpastatin (20 μM), a specific inhibitor of calpains, an overexpression of MDM2 and a decrease in the stabilization of p53 were obtained. This work shows a new pathway in the regulation of p53 in response to solar irradiation, where calpains first participate in the downregula
tion of MDM2 in the epidermis and subsequently contribute to a specific cleavage of p53 protein.

IL093 The Laboratory Opossum Model for UVB-Induced Skin and Eye Cancers
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Monodelphis domestica, the gray short-tailed opossum, is the only non-transgenic mammal known to be susceptible to UVB-induced melanoma in the absence of promoters or co-carcinogens. They also are highly susceptible to corneal cancer induced by UVB. Susceptibility to the corneal cancer is under genetic control; the estimated heritability of corneal cancer in the gray short-tailed opossum model is well suited for investigating the significance of dietary and other environmental factors that are hypothesized to increase or decrease the risk of melanoma or corneal cancer. Adult opossums to low dose UVB over 7–10 months produce melanocytic skin cancer, predominantly in samples excised from UV-exposed body sites. In this study we further investigated the induction mechanisms of CRBCs in the basal layer of human skin. Volunteers were irradiated with a chronic (0.3 MED daily for 5 days) and acute (1.5 MED) dose of UVB. Immediately after the irradiation or 14 days later skin biopsies were taken, skin sections were prepared and stained with CPD antibody. Though we found a great interindividual variability according to repair of UV damage, CRBCs were detected in the majority of the samples after chronic as well as after acute irradiation. If it turns out that the CRBCs are precursors of (non-melanoma) skin cancer cells, this would be very important for the development of a molecular epidemiology of skin cancer.

FC95 Interaction of CPT retaining basal cells after UV-irradiation of human skin – a first step of cancer initiation
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UV radiation is the most important factor responsible for skin cancer. However, the molecular and cellular mechanisms which cause UV-induced skin cancer still need to be explored in more detail to understand photocarcinogenesis. We recently published that chronic UVB-irradiation leaves Cytochrome-pyrimidine-dimer (CPT) retaining cells (CRBCs) in the epidermis of mouse skin which can be detected using a fluorescent monoclonal antibody against CPT. CRBCs were also found in human skin biopsies predominantly in samples excited from UV-exposed body sites. In this study we further investigated the induction mechanisms of CRBCs in the basal layer of human skin. Volunteers were irradiated with a chronic (0.3 MED daily for 5 days) and acute (1.5 MED) dose of UVB. Immediately after the irradiation or 14 days later skin biopsies were taken, skin section were prepared and stained with CPD antibody. Though we found a great interindividual variability according to repair of UV damage, CRBCs were detected in the majority of the samples after chronic as well as after acute irradiation. If it turns out that the CRBCs are precursors of (non-melanoma) skin cancer cells, this would be very important for the development of a molecular epidemiology of skin cancer.
FCO98
Macroscopic and microscopic fluorescence imaging of human bladder cancer using hypericin as a photosensitizer
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In this study we have investigated the use of hypericin as a fluorescent tumour marker and laser confocal microscopy as a diagnostic tool to aid the diagnosis of such cancers. In the cellular studies, we have compared two blader cell lines for the uptake and subcellular localization of hypericin. It was found that there was rapid uptake and clearance of hypericin and significant localization in mitochondria and lysosomes. The study also revealed that there was a time dependent increase in fluorescent intensity in bladder cells. Optimum localization was found to be 24 hours post drug incubation. In the clinical study, consisting of 30 patients, both white light and fluorescence cytoscopies were performed after hypericin instillation. Biopsies taken from the bladder regions were imaged using the confocal microscope. The order of fluorescence was observed to be as follows: normal < inflammation < grade 1 < grade 2 < CIS < grade 3. It was also discovered that the fluorescence intensity increased with the stage of the disease, thereby enabling the determination of the degree of invasiveness of cancer. This enables the use of hypericin as a prognostic marker and laser confocal microscopy as a tool to aid in diagnosis of bladder cancer.

TC102
Detection of precancerous and early cancerous lesions in the bronchi by fluorescence/reflectance imaging with a spectrally optimized system
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Fluorescence endoscopy is emerging as a useful tool for the detection of precancerous and early cancerous lesions. This approach is based on the fluorescence changes existing or induced between these lesions and their normal surrounding tissues. Unfortunately, many spectral features of interest in photodetection systems remain unclear and/or sub-optimal at the present time. Therefore, our goals are: 1) to conduct a comprehensive study of the autofluorescence spectroscopy of the human healthy, metaplastic, dysplastic and cancerous tissues in hollow organs, and 2) to evaluate the performances of fluorescence imaging apparatus resulting from this spectral study. These measurements were performed with a spectrally and intensity calibrated optical fiber-based spectrophotometer, covering excitation wavelengths ranging from 350 to 480 nm. Moreover, the absolute values of the tissue autofluorescence yield were determined. This study demonstrated that the excitation wavelengths yielding the highest contrasts in the tracheo-bronchial tree are between 400 and 480 nm with a peak at 405 nm. It was observed that the transition wavelength for bispectral fluorescence imaging systems is around 590 nm in this organ, regardless of the excitation wavelength. The order of magnitude of the autofluorescence brightness is stable in this organ as the excitation varies from 350 to 495 nm (on the order of 5 nW/mm x nm). We also found that the use of backscattered red light, instead of red autofluorescence, enhances the "lesion/normal" tissues contrast obtained with such imaging systems used in the bronchi by a factor 2. The clinical evaluation of a strongly simplified and optimized fluorescence imaging apparatus demonstrated that the positive predictive value can be significantly improved, when combining white light and autofluorescence bronchoscopy. Furthermore, the sensitivity is estimated to be twice higher in the autofluorescence mode than in the white light mode.

FCO99
Photodynamic detection of diseased sentinel lymph node after oral application of aminolevulinic acid (ala) in patients with breast cancer
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Background
In this study we evaluated the fluorescence intensity of metastatic sentinel lymph node (SLN) and metastatic axillary lymph nodes (ALN) compared to disease free lymph nodes in patients with breast cancer.

Material and Methods
11 patients received 30mg ALA/kg bodyweight orally three hours prior to surgery. The SLN was marked with Nanocil (R) and with Blue dye (R), tumor excision, excision of the SLN and an axillary dissection were performed. The operation site was illuminated with blue light (400nm) to obtain macroscopic tissue characterisation. Tissue samples were stored protected from light and analysed using a fluorescence microscope. The results were correlated with histopathology.

Results
A total of 7 primary tumors, 11 SLN and 8 ALN were analysed. Metastatic SLN demonstrated a statistically significant higher fluorescence intensity (FI) than non-metastatic SLN (2630 versus 526, p<0.0001). The FI of metastatic lymph node tissue and the primary tumour was significantly higher compared to the normal mammary tissue. In a few cases it was possible to recognize the metastatic SLN macroscopically with blue light.

Conclusion
Our study indicates, that PDD with ALA has a potential in the diagnosis and metastatic SLN macroscopically with blue light.

IL100
Autofluorescence as an intrinsic parameter for biological tissue characterisation
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Most biological components involved both in functional and metabolic processes (enzymes, flavins, lipopigments, porphyrins) and in histological tissue organization (constitutive proteins) act as fluorophores giving rise to a fluorescence emission (autofluorescence) that covers the visible range upon excitation in the UV-blue spectral region. Since autofluorescence emission is related to the nature, relative amount and spatial distribution of endogenous fluorophores, the occurrence of pathological conditions affecting histological and biochemical tissue features is expected to result in an alteration of the autofluorescence spectral properties. On this basis, autofluorescence represents an intrinsic parameter for in situ cancer diagnosis through a minimally, or non-invasive, real-time technique. Data concerning colon and brain tumors are presented. In the colon, spectrofluorometric analysis via endoscopy evidenced that both adenocarcinoma and carcinoma can be distinguished from normal surrounding tissues on the basis of the emission amplitude and of the spectral shape. Malignant and premalignant lesions can be discriminated one from another according to their spectral shape. In the brain, differences in spectral shape and emission amplitude were found between glioblastoma and healthy tissue (white matter, cortex), that provide useful information to guide brain tumor resection during surgical operation.

FC101
Autofluorescence of tumour tissue: prospects of optical biopsy
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The first autofluorescence results from natural tissue chromosomes corresponding to the absorbed light. Autofluorescence measurements found application in tumour diagnosis. It is proved that the increased tumour autofluorescence in the red spectrum region is related to endogenous porphyrins concentration, and that presents a reliable diagnostic tool. But in some cases primary tumour diagnostics might be complicated, as autofluorescence intensity of endogenous porphyrins in different segments of the same tumour varies several times or even could not be observed. C57BL/6J mice, bearing hepatoma A22 in the right hip and healthy mice were used in experiment. Tumour tissue ex vivo was squeezed between two glasses. The blue LED light (λw = 410 nm) was used for excitation. Evaluation of tumour morphology provided, that certain segments of intact tumour tissue indicate broad fluorescence bands in 620 720 nm spectrum scale, which can be ascribed to endogenous porphyrins. Increase of average porphyrin concentration in intact tumour correlates with tumour proliferation. With synchronous measurement of signal, which is characterized by the absence of endogenous porphyrins fluorescence bands and do not express long wave spectrum differences in comparison with healthy tissues of muscle and skin. Autofluorescence data provide possibility to discriminate particular morphology of malignant tissues.

FC103
Predicting the reaction of photosensitive patients to polychromatic light sources: A mathematical method based on monochromator testing
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1Department of Photobiology, University of Dundee, Dundee, United Kingdom, 2Systems Engineering, Brunel University, Uxbridge, United Kingdom.
Photosensitive patients are routinely tested with monochromatic radiation to determine the spectral nature of their photosensitivity. Results from these tests have been used to determine a complete action spectrum for individual patients' photosensitivity. None the less, from this action spectrum, an effective irradiance for a patient's photosensitivity can be calculated for any polychromatic light source. Comparison of this effective irradiance with the patient’s lowest minimum erythemal (or urticarial) dose allows us to predict the exposure time at which the patient will react adversely. This predictive method has been tested by irradiating photosensitive patients with polychromatic sources. Results to date show that the reaction time can be predicted to within an average of +/− 28% of the actual reaction time. The accuracy inherent in predictions appears to be greater for sources that consist of only a minor amount of UVB. Using this method, patients can be informed of the potential hazards of exposure to different light sources. This is especially relevant where patients may be sensitive to polychromatic sources such as theatre lights and could be aware of this fact in the event of any surgical procedure. This method may also be valuable for assessing the maximum tolerable light exposure for patients that have been given photosensitizing drugs.
Seeds accumulate different flavonoids that influence their quality and nutritional value. In arabidopsis, proanthocyanidins (PAs), also called condensed tannins, specifically accumulate in the seed coat, giving the mature seed its brown colour. Genetic, molecular, and biochemical analyses have been undertaken in different laboratories, in order to characterize enzymes and regulatory mechanisms involved in PA metabolism. To date, 22 two loci have been identified and, according to the abnormal pigmentation of mutant seeds, named TRANSPARENT TESTA 1 (TT1) to TT19, TRANSPARENT TESTA GLABRA 2 (TTG2), TTG2, and BANANAS (BAN). Half of them have been characterized at the molecular level. Interestingly, BAN encodes an anthocyanidin reductase that catalyses the first committed step to PA metabolism. We have shown that the activity of the BAN promoter is restricted to the inner integument, where PAs accumulate. This specific expression is regulated by at least four TT proteins i.e. TT2, TT8, TT16, and TTG1. Furthermore, TT2 and TTG2 are also involved in the overall regulation of PA accumulation. The function of these proteins in the regulation of BAN expression and/or seed coat development will be discussed. Last, genetic ablation of the endothelium confirmed these results and allowed to study its role during seed coat development.

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**IL104**

**Regulation and function of flavonoid biosynthesis in arabidopsis seed**

J. Lepiniec1, A. Baudry2, M. Caboche1, I. Debrasouq1, N. Nesi3, L. Poure1, J. M. Routaboul4, 1INRA, Versailles, France, 2INRA, Rennes, France.

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**IL105**

**Regulation of Flavonoid Biosynthesis Genes and Aspects of UV Protection**

L. Jenkins

University of Glasgow, Glasgow, United Kingdom.

Flavonoids are phenolic plant secondary metabolites produced from a branch of the general phenylpropanoid pathway whose first enzyme is chalcone synthase (CHS). Flavonoids have a range of important functions, including as UV-absorbing protective pigments. Experiments using Arabidopsis mutants with elevated or reduced levels of flavonoids and sinapic acid esters indicate that both classes of compounds contribute to UV protection. The expression of genes encoding enzymes of the flavonoid biosynthesis pathway is regulated at the level of transcription by a range of endogenous environmental signals. We have started to define the complex network of interacting pathways that regulate these genes in Arabidopsis leaf tissue, and produced a model of the different light signalling pathways that regulate the CHS gene (Wade et al. 2001, Plant J. 25, 675–685). We are attempting to identify signalling and effector components involved in these responses using different approaches. We have isolated several regulatory mutants using CHS promoter-reporter screens in Arabidopsis. In addition, we are studying the regulation of transcription factors that control expression. The basic leucine zipper transcription factor HY5 is required for light induction and specific MYB transcription factors are also involved.

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**IL106**

**UVR8 and direct UV-B signal transduction**

D. J. Kieber-Emmons

University of California, Davis, Davis, CA, United States.

To further our understanding of how plants defend against the harmful effects of ultraviolet light, we characterized an Arabidopsis thaliana mutant hypersensitive to UV-B. This mutant, uvr8-1, contains a single recessive mutation at the bottom of chromosome 5. Fine-scale mapping localized uvr8-1 to a 21 kb locus, containing 5 predicted open reading frames. Sequencing of this entire region revealed that the uvr8-1 allele contains a 15’ nucleotide deletion in a gene similar to the human guanine nucleotide exchange factor RCC1 (regulator of chromatatin condensation). This mutation reduces the UV-B-mediated induction of flavonoids and Blocks chalcone synthase mRNA and protein induction. In contrast, uvr8-2 has enhanced induction of PR1 and PR5 proteins in response to UV-B, an indication of increased UV-B injury. These results suggest that UVR8 acts in a UV-B signal transduction pathway leading to induction of flavonoid biosynthesis. We will present results on our analysis of the predicted interaction between UVR8 and the RAN small G-protein as well as preliminary results on using yeast-two hybrid screens to analyze the UVR8 mediated UV-B signal transduction pathway.

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**IL107**

**Nutritional enhancement of plants by manipulation of secondary metabolism levels**

M. E. Verhoeven

Lasenber R&D, Colworth, Bedford, United Kingdom.

Secondary metabolites such as terpenoids and flavonoids are implicated in a diverse range of functions in plants such as the attraction of insect pollinators and seed dispersing animals, protection against overeating by herbivores, plant growth and fertility, plant symbiosis, ancillary pigments in photosynthesis, etc. These natural compounds are also of great importance to the human food chain as they make major contributions to the colour, taste and flavour of foods and drinks. In addition many secondary metabolites have biological activities relevant to human health. Examples of terpenoids with health benefiting properties include beta-carotene which is an important source of vitamin A for humans; lycopene, which is thought to protect against prostate cancer; and the phytoestrogens, consumption of which can lower cholesterol levels in humans. In the area of the flavonoids, the flavonol quercetin has been shown to significantly reduce elevated blood pressure in spontaneously hypertensive rats. Flavonoids are also thought to confer protection against cancer, dementia, viral infection etc., although more research is needed in these areas. It is therefore not surprising that a number of groups have developed strategies to enhance levels of beneficial secondary metabolites in plants. Examples to be discussed include the meeting’s discussion on the manipulation of flavonol and phytosterol levels by means of genetic manipulation.

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**IL108**

**Fluorescence correlation spectroscopy unravels signaling dynamics in the apoptotic pathway**

J. Woehrle

Physikalisches Institute, Stuttgart, Germany.

Signaling networks in cells and organs show a complicated time space pattern. One of the most pressing aims of modern life cell imaging is thus to provide reliable information on where and when certain signaling molecules interact. Quantitative microscopy is what is mostly needed in this respect. Fluorescence correlation spectroscopy (FCS) is an emerging technique in life cell imaging which allows for quantitative imaging with time resolution well beyond the time scale of most other imaging techniques. Since it is based on ultrasensitive fluorescence detection which allows for the observation of single molecules it complements existing techniques of cellular imaging towards the early events of signaling. The talk will discuss the application of FCS on the apoptotic signaling cascade. The clustering of receptors upon ligand binding initiates the intracellular signaling cascade. FCS gives a quantitative picture of this receptor clustering and subsequent events in the signal transduction cascade. The results are complemented with wide field images of single diffusion receptors in cell membranes.

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**IL109**

**Laser-Assisted Fluorescence Microscopy in Cell Biology and Photobiology**

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The use of lasers in fluorescence microscopy offers numerous advantages, e.g. coherence, focusing abilities, small spectral bandwidth as well as the possibility to create ultrashort light pulses. Focusing of laser beams is used in laser scanning microscopy, total internal reflection fluorescence microscopy (TIRFM) and laser micromanipulation, whereas short laser pulses are applied for detection of fast kinetics, time-gated fluorescence spectroscopy and fluorescence lifetime imaging (FLIM). An overview on these techniques, their advantages and applications to cell markers and photosensitizers is given.

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**IL110**

**Fluorescence lifetime imaging**

P. M. W. French

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Fluorescence lifetime imaging (FLIM) is a functional imaging methodology that is currently experiencing a rapid increase in uptake, due to its unprecedented specificity and sensitivity and its immunity to intensity artefacts. It is applicable to imaging intracellular function, e.g. using fluorescence resonant energy transfer (FRET) technique and can provide information, not only concerning the localisation of a specific fluorophore, but also about the local fluorophore environment. It may be implemented in scanning confocal or multi-photon microscopes, or in wide-field microscopes and endoscopes. When applied to tissue autofluorescence, it reveals intrinsic excellent contrast between different types and states of tissue. This talk will review our recent progress in developing and applying FLIM technology for microscopy and endoscopy.
FC111 Fluorescence lifetime imaging of photosensitizer metabolites using ps diode lasers and time-correlated single-photon-counting in laser scanning microscopes
A. Rueck, F. Dolp, E. Hasebrock, C. Scalfi-Hopp; ILM, Ulm, Germany
A setup consisting on a laser scanning microscope (Zeiss, Germany) equipped with appropriate detection units was developed for fluorescence lifetime imaging (FLIM) for on-line detection of structural changes of various biomolecules. Short-pulse excitation was performed with diode lasers which emit pulses at 398 nm and 440 nm with 70 ps pulse duration (PicoQuant, Germany). The laser diodes were coupled to the laser scanning microscope via external coupling units and appropriate beam splitters. An ultrafast photomultiplier was used together with a time-correlated single photon counting module (TCSPC; SPTR-730, Becker & Hickl, Germany) to determine the fluorescence lifetime of different metabolites of photosensitizers with subcellular resolution and to record lifetime images (t-mapping) (1). Especially, we will discuss the time-resolved fluorescence characteristics of 5-ALA (5-aminolevulinic acid) induced protoporphyrin IX (PPIX) and other metabolites as well as the lipophilic derivative 5-S-AHE (5-aminolevulinicacid hexylester). In principle three different lifetime regions could be observed in the cells. Regions, correlated with lifetimes around 10 and 12 ns were found in the mitochondria and plasma membrane of the cells and attributed to PPIX, whereas a lifetime around 6 ns possibly coincides with metabolites. In the case of 5-S-AHE the lifetime of PPIX was even longer, which could be due to different subcellular localization. During illumination the component with the longer lifetime completely vanished, whereas the shorter lifetime was retained. Because the photodynamic effects can be correlated with the lifetime of the excited states it seems that FLIM, using ps diode lasers and TCSPC is a valuable method to selectively identify and localize the photodynamically active photosensitizer.

(1) M. Kress, Th. Meier, R. Steiner, F. Dolp, R. Erdmann, U. Oertlm, and A. Rueck
Time-resolved microspectrofluorometry and fluorescence lifetime imaging of photosensitizers using ps pulsed diode lasers in laser scanning microscopy,

IL112 Time-resolved optical mammography: from instrumentation development to clinical application
E. Taroni, A. Pifferi, L. Spinelli, A. Torricelli, G. Danneskin, R. Cudaback; Politecnico di Milano-INFM and IFN-CNR, Milan, Italy, IFN-CNR, Milan, Italy, Clinica S.Pio X, Milan, Italy
The first time-resolved optical mammography operating even at wavelengths longer than 900 nm (683, 785, 913, and 975 nm) was developed. Interpretation of time-resolved transmission data allow the assessment of both the absorption and the scattering properties that are related, respectively, to the composition and structure of tissue. In particular, from the optical properties at 4 wavelengths, the concentrations of oxy- and deoxy-hemoglobin, scatterers, and the effective abundance of water and lipids can then be estimated, together with oxygen saturation in tissues. The instrument is presently used in a European multi-centric clinical trial to test the diagnostic potential of time-resolved optical mammography (E. Taroni, personal communication). The results showed that 5 patients out of 126 patients up to April 2003. Late gated intensity and effective scattering images are routinely adopted for the detection and discrimination of breast lesions. Moreover, the perturbation analysis is applied for the quantification of the optical properties and physiological parameters of the detected lesions.

IL114 ALA and ester-ALA-PDT – basic principles and molecular mechanisms of action
B. Kramer, T. Verwanger, R. Sanovic; Institute of Physics and Biophysics, Salzburg, Austria.
He photosensitizer protoporphyrin IX (PPIX) is an intermediate in the heme biosynthetic pathway which is overproduced by cells after external administration of the precursor 5-aminolevulinic acid (ALA), endogenous PPIX, preferentially accumulated in tumors can be used as a highly reliable fluorescence marker for photodagnosis as well as for treatment of superficial tumors of the skin and inner organs. The poor penetration of ALA into tissue and across cell membranes, when applied topically, could be improved by the development of ala derivatives, which are taken up by the cell via another mechanism than ALA. It seems that ALA-esters are more efficient in PPIX production and show less systemic effects after local application than ALA. PPIX is accumulated in ALA with a higher rate in epidermal vs. untreated cells and localizes in membrane systems and mitochondria. By irradiation with light of a wavelength of 635 nm, necrosis or apoptosis is induced as photodamage, dependent on treatment dose and sensitizer localization. On the molecular level changes in the expression of genes involved in apoptosis and dna repair, of immediate early genes, heat-shock-protein 70 and of genes involved in proliferation were detected. Acknowledgements: study supported by fwp (project no: p15143)

IL115 Clinical experiences with 5-ala-pdt in urology and neurosurgery
B. Baumgartner; Laser-Forschungs labor, Munchen, Germany.
First clinical experiences on whole bladder photodynamic therapy after intravesical administration of 5-aminolevulinic acid (5-ala) using a white light source are reported. The study was designed to define the optimal target group of patients for this therapy and is considered as a basis for long term and multicenter studies. Whole bladder photodynamic therapy was performed with 100 j/cm2 white light 2 to 4.5 hours after intravesical administration of 17% 5-ala in 12 patients with recurring, multifocal stage pTa grade I – III urothelial tumors and carcinoma in situ. Immediately after whole blad- der irradiation, histological examination of biopsies taken from flat sus- picious lesions showed no viable cells, while remnants of malignant cells were found in papillary tumors. At a median follow-up of 18 months (range 3 to 25), 3 of the seven patients with carcinoma in situ and two of the four patients with papillary tumors were disease free. Conclusion: The preliminary data show that 5-ala pdt with a white light source is effective in destroying flat malignant lesions in the bladder like carcinoma in situ. The procedure is easy to perform and is not associated with any major side effects. A second clinical trial with 5-ala in urology was performed on human prostate cancer, following animal experimental studies. First the localization of 5-ala induced protoporphyrin IX in the prostate cancer is investigated. For that purpose 14 patients received 20 mg/kg bw 5-ala orally prior to a rad- ical prostatectomy. As shown by fluorescence microscopy, fluorescence was observed exclusively in carcinoma cells, while the epithelial cells and the stro- matal tissue showed no fluorescence. Afterward the tissue was fixed in paraformaldehyde and dehydrated in alcohol and xylene. Paraffin sections were made and stained with hematoxylin and eosin. An irradiation of 250 cm2 of laser light at an irradiance of 0.5 cm2 was applied. 6 weeks after postoperative the psa values were reduced by 20% up to 70%. With regard to the side effects no patient complained about incontinence or dysuria after pdt. This demon- strates that interstitial pdt of prostate cancer by means of 5-ala induced ppx is a safe and simple procedure. Furthermore investigations concerning light deliver- y and dosimetry are warranted to obtain a complete and curative treatment of prostate cancer. Since selective accumulation of protoporphyrin IX in malignant glioma tissue after application of 5-ala has been proven on hun- dreds of patients the photosensitising potential of ppx was clinically investi- gated for pdt of malignant glioma. Study aim was to determine efficacy and side effects of phototheraphy of malignant gliomas following administration of 5-ala. In a prospective study seven patients with recurrent glioblastoma multiforme received 20 mg 5-ala/kg bw orally 3 hours prior to induction of anesthesia. resulting tissue fluorescence was used for tumor resection with a modified surgical microscope (zeiss nc 4!l). Non-resectable tumor was irradi- ated focally by laser light (&895; &633nm) via a microlens system. Following fluorescence guided operation and focal pdt, one patient develope- ed an isolated lateral ventricle. he recovered completely after shunting. A causal relationship to the pdts was considered unlikely. No further adverse events were encountered. early mri scans were devoid of residual enhancing tumor in all patients. in conclusion 5-ala pdt for malignant glioma appears safe. Since a definite phototoxic effect was verifiable by biopsy, this mode of treatment appears promising and should be explored further.

IL116 ALA and ester-ALA-PDT for oncologic indications in dermatology
A. R. Oseroff; Roswell Park Cancer Institute, Buffalo, NY, United States.
Utilization of topical PDT for cutaneous malignancies continues to grow, with mal geographic approaches to treatment conditions. The use of choices of light dose and fluence rate, illumination wavelengths, skin preparations and the number of sequential treatments. It is difficult to optimize these parameters in a clinical trial. Real-time optical measurements of photobleaching kinetics, and possibly of hemoglobin spectra, can help guide the choice of illumination conditions and drug levels to avoid photodynamic oxygen depletion and to deliver adequate light. Recent successes with pulsed 595 and 595 nm light sources and low fluences focus attention on cellular injury and the discharge of host inflammatory and immune systems. Taken together, the data may lead to new approaches, and to rational combinations of PDT with other agents.
IL117 Photodynamic therapy for non-oncologic indications in dermatology
R. Szemies, S. Karren
Department of Dermatology, Regensburg, Germany.

Topical and systemic photodynamic therapy (PDT) is a well established treatment for various benign cutaneous and epithelial skin disorders, but it is evident that also inflammatory diseases of the skin and virus induced lesions can profit from PDT, depending on the light dose applied and either cytotoxic effects resulting in tumor destruction or immunomodulatory effects resulting in improvement of inflammatory conditions occur. Patients with localized scleroderma and sarcoidosis of the skin had been unresponsive to various treatments including puv-a or bath-puva therapy, respond very well to repeatedly performed topical ala-PTx. In contrast to puv-a therapy, no carcinogenic potential is being discussed for PDT. Also hpi-induced skin lesions might provide a possible indication for topical ala-PTx. The rapidly proliferating cells in viral acanthomas accumulate ala-induced ppx selectively when compared to the surrounding non-neoplastic cells. The efficacy of topical ala-PTx in the treatment of recurrent foot and hand warts has been shown in a placebo-controlled randomized double-blind trial. Furthermore, case reports describe a good response of other virus induced lesions, including carcinoma in situ. Therefore improved detection and treatment is still needed to demonstrate more fully the effectiveness of PDT for inflammatory skin diseases.

IL118 S-Aminolevulinic Acid-Containing Dendrimers and Other Derivatives as Prodrugs for Photodynamic Therapy: Synthesis and Biological Evaluation
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2Inst. Of Oral and Maxillofacial Surgery, University of Tokushima, Tokushima, Japan.

We have been investigating new delivery methods for S-aminolevulinic acid (ALA) which is the precursor of protoporphyrin IX (PpIX) for use in photodynamic therapy (PDT). MACrocyclisations of conjugated with a number of drug molecules to improve for use in PDT delivery the produg following enzymatic intracellular drug release. Using a convergent growth approach, a series of novel S-aminolevulinic acid (ALA)-containing dendrimers have been synthesized. The ALA residues were conjugated using ester linkages to a branched dendron covalently joined to a di- or tridentate aromatic, or tridentate aliphatic core through amide bonds. Two first generation dendrimers, bearing either 6 or 9 ALA residues, were synthesized by attachment of a tri(bis-protected ALA)-containing wedge which were cleaved later and isolated as ALA-TEA dendrimer salts. A second generation 18-ALA-containing dendrimer was also synthesized. These ALA-TEA dendrimers contained conjugated using ester linkages to a branching dendron coupled to a di- or tridentate aromatic, or tridentate aliphatic core through amide bonds. Two first generation dendrimers, bearing either 6 or 9 ALA residues, were synthesized by attachment of a tri(bis-protected ALA)-containing wedge which were cleaved later and isolated as ALA-TEA dendrimer salts. A second generation 18-ALA-containing dendrimer was also synthesized. These ALA-TEA dendrimers were tested as prodrugs for PDT and their behavior following PAR 212 keratinocytes were irradiated at 6 s 3 nm following 2,4 and 6 hrs incubation. The most effective in terms of PpIX accumulation and PDT effect was the 18-ALA dendrimer. For comparison smaller trimeric ALA moieties have also been prepared and mechanistic studies on cellular uptake using fluoresein-labelled dendrimers are in progress.

IL119 Hexyl aminolevulinate (hal) for diagnosis and treatment of bladder cancer
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Photoclere ASA, Oslo, Norway.

Hexyl aminolevulinate, hal (hexvix®, photocure asa), is a novel potent photo sensitiser giving adequate porphyrine concentrations after instillation into the bladder of as little as 85 mg for one hour. hal selectively accumulates in malignant cells providing high porphyrine concentration useful for both fluorescence diagnosis as well treatment of cancer. Bladder cancer, most commonly arising as transitional cell carcinoma of the urinary tract, is one of the cancers with highest prevalence. The high recurrence rate that might attain 70% is often attributed to an incomplete resection of all present tumour areas and insufficient capability to recognize flat lesions including carcinoma in situ. Therefore improved detection and treatment is required. The technology has been optimised considering the clinical, chemical as well as the technical aspects. Therefore hal fluorescence cystoscopy now offers to urologists a reliable tool in order to improve management of superficial bladder cancer. More than 800 patient have been enrolled in four multi centre studies, for the detection of bladder cancer, taking place in more than 40 urology clinics in Europe and north America. In the studies patients with large bladder tumours were included. HAL Fluorescence cystoscopy was performed with a d-light (kari storz, Germany) allowing for both white light and blue light (375–440 nm) bladder inspection. The studies showed hal significantly increased detection of tumours both on a patients and tumour basis, and also showed that intended patient management would change. Extension of this methodology towards photodynamic therapy (PDT) will be one of the major milestones in the future, and clinical trials have been initiated.

IL120 PAR and UVBR Induced oxidative stress and related protection mechanisms in Antarctic marine microalgae
A. G. Bunt1, B. L. van den Enden1, S. W. Wright1, A. D. Davidson2
1University of Groningen, Haren, Netherlands, 2Australian Antarctic Division, Kingston, Australia.

Recent studies suggest that in situ UVBR (ultraviolet-B radiation, 280–315 nm) may cause viability loss in Antarctic marine microalgae, which could be related with the formation of highly reactive oxiradicals, causing oxidative stress. Nothing is known about PAR-related oxidative stress in Antarctic microalgae. In the present study we acclimated four Antarctic microalgal species (Phaeosycystis antarctica, Polanealia glacialis, Chaetoceros richardii and Pyramimonas gelidicola) to five PAR levels. After acclimation samples were taken for xanthophyll pigment analysis (HPCL), UV absorbing compounds, SOD and malondialdehyde (MDA), the latter used as a general indicator of oxidative stress. Subsequently the cultures were exposed to an identical UVBR treatment, after which further MDA accumulation and UVBR induced DNA damage was determined. Preliminary results show a very strong PAR related induction of UV absorbing compounds and xanthophyll pigments. Oxidative stress was highest in high PAR acclimated cells despite elevated levels of SOD, UV absorbing compounds and xanthophyll pigments under these conditions. The UVBR treatment caused further MDA accumulation in most cultures, but specifically in those cultures which were acclimat ed to high PAR. The present results suggest that acclimation to high PAR does not necessarily offer adequate protection against oxiradicals generated by UVBR.

IL121 Biological UV dosimetry
F. Seth; A. G. F. Duek; DLR Institute of Aerospace Medicine, Radiation Biology, Köln, Germany.

The determination of the effects of changes of environmental UV radiation on critical processes in the biosphere requires accurate and reliable monitorings systems that weight the spectral irradiance according to the biological responses under consideration. The need for a biological weighting of solar UV irradiation derives from the highly wavelength-dependent sensitivity, expressed as the so-called action spectrum, of biological systems. Biological UV dosimeters, that weight directly the incident UV components of sunlight in relation to the effectiveness of the different wavelengths and the potential interactions between them, can complement physically based UV measurement systems. Up to now several UV-dependent endpoints in biomolecules (e.g. thymine dimer (TD), DNA, pre-vitamin D3, bacteriophages (e.g. T7), bacteria (e.g. E. coli, B. subtillis) and cultured eukaryotic cells have been suggested as sensing elements in biological UV dosimeters. One example is the DLR-Biofilm consisting of immobilized spores of the bacteria B. subtillis as UV sensor. It weights per se the incident UV radiation according to its DNA-damaging effectiveness. Examples for the application of the DLR-Biofilm technique for personal UV dosimetry and for the measurement of the biologically weighted irradiance of the sun and also of artificial UV sources will be discussed.

IL122 HFR1, a putative bHLH transcription factor, mediates both phytochrome A and cryptochrome signalling
C. Falkhauser, P. D. Duek; University of Geneva, Geneva, Switzerland.

Plants are very sensitive to their light environment. They use cryptochromes and phytochromes to scan the light spectrum. Two of these families of photoreceptors mediate a number of similar physiological responses. The putative bHLH transcription factor HFR1 is important for a subset of phytochrome A (phyA) mediated light responses. Interestingly, phyA and HFR1 alleles also have reduced de-etiolation responses, including hypocotyl growth, cotyledon opening and anthocyanin accumulation, when grown in continuous far-red light. This phenotype is particularly apparent under high fluence rates. The analysis of double mutants between hfr1 and different blue light photoreceptor mutants demonstrates that, in addition to its role in phyA signalling, HFR1 is a component of cryptochrome 1 (cry1) mediated light signalling. Moreover HFR1 mRNA levels are high both phyA and cry1. www.ESP2003.org 60

Abstracts
IL123 
Bacteriochlorophytes and regulation of the synthesis of the photosynthet-
ic apparatus in Rhodopseudononas palustris 
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Recent biochemical and genetic studies have demonstrated the occurrence of 
phototransformable protein complexes in the photosynthetic and non-photosynthetic bacte-
ria but no clear function has been assigned to these light sensors. We have 
discovered a new bacteriochlorophytochrome located downstream from the photo-
synthesis gene cluster in a symbiotic Bradyrhizobium strain and for the closely
related species Rhodopseudomonas palustris. Unlike other (bacterio)phy-
chrotochromes, the C-terminal domain of this new bacteriochlorophytochrome contains 
no histidine kinase features. The absence of histidine kinase domain suggests a tool and simplified regulatory system involving direct protein-protein 
interaction with no phoshophorylation cascade. The chromophore of this bacterio-
chlorophytochrome is the simplest linear tetrapyrrole, biliverdine. Using a combina-
tion of biophysical and biological approaches we show that the synthesis of the entire photosynthetic apparatus is under the control of this new bacte-
riophytochrome in the presence of oxygen. This light regulation, via a bacte-
riophytochrome, is of prime importance for the energetic status of the bacte-
ria since it allows their rapid switch from dark heterotrophy to the more ener-
gievably favourable photoheterotrophic growth.

FC124
UV protection and shade structures
University of Southern Queensland, Toowoomba, Australia.
Broadband field measurements were conducted beneath three different 
sizes of public shade structures at a sub-tropical Southern Hemisphere site for 
relatively clear skies and for a changing solar zenith angle (SZA) of 13° to 76°.
These data were compared to the diffuse UV to quantify the relationship 
between diffuse UV and the UV in the shade of the structures. On the hori-
zontal plane, the ultraviolet protection factors (UPF) for the shade structures 
ranged from 1.5 to 18 for a decreasing SZA. The data from this research is sig-
nificant, because it shows that as the SZA of the sun increases so does the rel-
ative proportion of scattered UV beneath the shade structures which in turn 
decreases the shade structures UPF. In Australia, erythemal UV in full sun can reach 
levels of approximately 2.5 MED/h or more in the middle of the day dur-
ing winter. Therefore, it is necessary for people that live in similar latitudes to 
minimise UV exposure in all climatic conditions throughout the year. Based 
on this research, a standard for reporting the UV protection provided by 
shade structures is essential for the public to make an informed decision on the 
efficacy of particular structures in reducing personal UV exposure.

FC125
Photochemistry and photoactivity of 1-aminopyrene
Jackson State University, Jackson, MS, United States.
Aromatic amines are a class of mutagens in the environment. 1-Aminopyrene 
(1-AP) is a metabolite of 1-nitropyrene (1-NP). Upon light irradiation, 1-AP in 
aqueous solutions transforms into photoproducts: 1-hydroxyaminopyrene (1-
HOP), 1-nitropyrene (1-NP), 1-NP, 1-amino-x-hydroxypyrene, and three 
covalent dimers. The phototransformation half-lives depend heavily on the 
solvent used and co-existing chemicals: NaCl, MgCl2, histidine, dithiothreitol, 
and DNA. The progressive oxidation products from amino to hydro-
yamino, nitroso, and nitro is the reverse of the enzymatic reduction of the 
nitro group in 1-NP in living systems. Using the Mutatox test system, it was 
found that the lowest observable genotoxic concentrations for 1-AP, 1-AP 
photoproducts, 1-NP were 1.25, 10, and >10 M in direct medium and >10, 5, 
and 0.625 M in s-9 medium, respectively. The 1-AP photoproduction mixture is 
more genotoxic than 1-NP in direct medium, but in s-9 medium, it is less 
genotoxic than 1-NP and more genotoxic than 1-AP. This suggests that 1-NP 
is not responsible for all the mutagenicity of the photoproducts. Irradiation of 
1-AP together with DNA leads to the formation of covalently bound 1-AP-
DNA adducts. Acknowledgements: Grants from National Institutes of Health: 
SCORE 506 GM08047 and RCMI S212RR12459, and US Army Research Office: 
DAAD 19-01-1-0733.

FC126
Impact of riboflavin on photo-transformation and photo-toxicity of selected 
environmental contaminants in water
L. Chung, K. Zeng, H. E. Glover;
Jackson State University, Jackson, MS, United States.
In our laboratory, sensitized effect of riboflavin on the photosynthetic rate of 
2,4,6-trinitrotoluene (TNT), atrazine, and selected polycyclic aromatic hydro-
carbons was studied. Riboflavin (1μM) significantly enhanced photo-transfor-
mation rate of TNT (10 mg/L) in a natural water. HPLC assay result indicates 
that transformation of TNT in the presence of riboflavin undergoes different 
pathways. At higher concentration (100 μM) riboflavin significantly enhanced 
photolysis rate of atrazine (10 μg/L) and more than 80% of atrazine in a nat-
ural water sample was depleted in 72 hours. Atrazine transformation rate 
was faster in the natural water samples. Assay by GC-MS and HPLC indicates 
that dealkylation and alkylation reactions were involved in the degradation 
pathway of atrazine. Plate counting indicates the occurrence of photoinduced 
cytotoxicity during transformation process and that is of concern to the 
microbial assemblages needed for complete remediation. The photo-trans-
formation half-lives of 6-aminochrysene and benzo[a]pyrene-7,8 were also 
shortened significantly in the presence of riboflavin (10-100 μM) in 10 mM PBS 
solution (pH 7.0). However, no significant influence on 1-nitropyrene pho-
tolysis was found under the same conditions. This research was funded by 
grants (1) NIH-RCMI G12RR13459-03, (2) NIH-MBRS S06GM08047, and (3) 
U.S. Department of the Army DAAD 19-01-1-0733 awarded to JSU.

IL128
Ultraviolet radiation suppression of recall immunity in humans: nitric oxide, 
DNA damage and UVA
C. M. Halliday, I. M. Kuchel, T. S. C. Poon, S. N. Byrne, R. S. Barnetson;
University of Sydney, Sydney, Australia.
We examined the mechanism by which solar-simulated ultraviolet radiation 
(ssUVR) suppresses recall responses to nickel in allergic humans. L-NMMA 
inhibited nitric oxide (NO) production and T4N5 liposomes containing T4 
endonuclease V enhanced DNA repair. Sub-erythemal ssUVR caused a dose-
related local immunosuppression. L-NMMA and the liposomes protected the 
nickel reaction, suggesting that NO and DNA damage are mediators of UV-
induced immunosuppression in humans. Immunostained biopsy sections 
suggest that NO and DNA damage are mediators of UV-induced immunosuppression in humans. Immunostained biopsy sections 
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suggest that NO and DNA damage are mediators of UV-induced immunosuppression in humans.
Update on UV-induced suppressor/regulatory T cells

Kinki University School of Medicine, Osaka-Sayama-city, Japan.

UV light, in particular the middle wavelength range called UVB is one of most hazardous environmental impacts affecting human skin. Radiation of UVB on human skin causes various health disturbances, such as premature aging, inflammation, oncogenesis and immune suppression. Among them our group has dealt with UV-induced antigen specific immune tolerance, since the exact understanding of this process may be a lesson to bridge clinical and basic knowledge about pathogenesis of allergic skin diseases. Generation of T suppressor/regulatory cells (Ts/r) plays a pivotal role in this process. Insufficient antigen presentation involving B7/2/CD28 is supposed to result in tolerance. In this context, it is worthy to note that ligation of B7 with CTLA-4, another B7 ligand than CD28, leads to expansion of Ts/r, thereby inducing tolerance. Employing an animal model of UVB-induced local immune suppression of contact hypersensitivity responses, we currently could show that denit-2, a receptor for C-type lectin and expressed on Langerhans cells (LC), play a pivotal role in the induction of tolerance. Importantly, functional neutralization of CTLA-4 or denit-2 in vivo breaks already established UV-induced tolerance, indicating their important role in this process and suggesting the possible usefulness of the in vivo blockage of these molecules to break pathogenic tolerability. Although details in immune mechanisms in this process gradually become disclosed for current decades, there are still numbers of unanswered questions, i.e. is UV-induced tolerance central or peripheral tolerance? Or does only blockage of B7/CD28 lead to tolerance? During my talk these issues are also addressed.

IL130

UV-induced immune modulation in humans – effect of skin type

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Within healthy, white-skinned Caucasians, susceptibility to UVB-induced immunosuppression is skin type-dependent. Skin types I/II are 2–3 fold more susceptible to immunosuppression than skin-types III/IV, for an equivalent MED challenge. This susceptibility to immunosuppression is associated with enhanced release of cytokines (including TNF and IL-10) in vivo, suggesting that a general mechanism for the induction/control of cytokine release differs in the two skin-type groups. DNA photo-lesions initiate immunosuppression and cytokine release in experimental models, however, we found that global DNA damage and repair was similar in both skin-type groups. In addition, we found no skin-type association with dermal mast cell numbers that are a source of TNF in UVB-exposed skin. Candidate gene analysis of several gene polymorphisms that are important in cutaneous immunity and inflammation, including those encoding cytokines, adhesion molecules, chemokine receptors and others did not show a skin-type association. The expression of many of these genes are controlled by the transcription factor NF-kappaB. Our recent work indicates that NF-kappab activation is greater in skin types I/II than III/IV. We also find that cis- but not trans-urocanic acid stimulates NF-kappaB activation. Thus skin-type differences in immunomodulation and susceptibility to immunosuppression may be due to differences in NF-kappaB response.

FC132

The Light-Activation of Human T-Cells

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The correct functioning of the immune response depends upon interactions occurring at the right times and locations. The remote control of such interactions would have many fundamental and clinical applications. We here employed light as the remote influence to exert such control. We have previously demonstrated the regulation of antibodies by light (Self & Thompson, 1996). An antibody is reversibly inhibited by conjugation with light-sensitive (2-nitropheneryl)ethanol – NPE) groups. It is possible to abrogate the antigen-binding activity of an antibody until exposure to UVA light whereupon its activity is restored. We here apply this technology to the remote regulation of human T-cells. An anti-human T-cell antibody (OKT3) which both binds and activates human T-cells is employed. Its activity is greatly reduced by NPE conjugation as monitored by T-cell (CD3 + cell line H9) binding, IL2 production and CD69 elaboration. T-cell activation is restored very simply by exposure to light. Thus the possibility now exists of being able to direct the activity of the immune system, both when and where in the body it is necessary by light. Many applications of this fundamental technology to the control of biomedical systems are envisaged.


FC133

Systemically immunosuppressive doses of solar-simulated UV activates lymph node B cells so that they become the dominant antigen presenting cell in vivo

S. M. Dyring, N. Spinks, G. M. Halliday; University of Sydney, Sydney, Australia.

Small, sub-erythematous doses of ultraviolet (UV) radiation significantly suppresses systemic immunity in mice and humans. To explore the cellular mechanisms of this immunosuppression, we exposed C57BL/6 mice to 3 consecutive doses of solar simulated UV known to cause over 50% systemic immunosuppression (1820 mJ/cm2). Three days later, the draining lymph nodes were analysed by flow cytometry. While the percentage of T cells and dendritic cells (DC) decreased after UV, the relative ratios of the different subsets was unaltered. The overall percentage of B cells was unaltered by UV, but they were more activated, with a significant proportion of B cells expressing 8 fold higher levels of MHC II and B220. Lymph node DC and B cells were purified from both UV-irradiated and un-irradiated control mice and conjugated to TNP. These cells were then i.v. transferred into naïve syngeneic hosts to assess their ability to induce contact sensitivity. DC activated TNP-specific T cells, while B cells did not. Moreover, transfer of B cells from UV-irradiated hosts together with DC from control mice resulted in immunosuppression. These results show that UV activates B cells and switches the dominant APC from a DC to a B cell such that suppression, rather than activation occurs.

FC134

Proinflammatory platelet-activating factor (PAF) pathway involved in psoriasis-UV induced immune suppression

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We hypothesized that PAF (a phospholipid proinflammatory mediator) may be involved in PUV-induced immunosuppression, similar to what occurs after UVB exposure (Walterscheid et al, 2002). The intraperitoneal injection of both a specific PAF receptor antagonist (PC4248) and a selective COX-2 inhibitor (SC236) completely abrogated topical 8-MOP+UVA-induced immunosuppression in C57H/HsnJ mice in the model of delayed-type hypersensitivity to C. albicans. Interestingly, when phosphatidylcholine (PC) with or without 8-MOP was UV-irradiated ex vivo in order to produce PAF-like molecules, immunosuppression resulted in mice after injection of both UV-irradiated, 8-MOP-treated or untreated PC solutions. This result contrasted the in vivo situation, in which exposure to UVA alone never resulted in immunosuppression. The injection of PC4248 or SC236 reduced PUV-induced IL10 upregulation in the skin and IL10 antibody administration led to abrogation of PUV-immunosuppression. This indicates that activation of the PAF pathway and the downstream production of regulatory cytokines are crucial events in PUV-induced immunosuppression. However, the discrepancy in the outcome of the "ex vivo-to-in vivo PC experiment" compared to the pure in vivo situation suggests that additional molecular effects in addition to PAF generation may be necessary for PUV-induced in vivo immunosuppression.
The effect of low doses UVA-1 radiation on immunoglobulin production by activated B-lymphocytes

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Treating patients with systemic lupus erythematosus (SLE) with low doses of UVA-1 radiation resulted in improvement of various parameters of disease activity. Anti-double stranded (ds)DNA and anti-Sjögren-syndrome (SS)-A lites in patients decreased (Polderman et al., Ann Rheum Dis, 2003). To get a better comprehension of the working mechanisms of UVA-1 radiation in SLE patients, we investigated the effect of low doses UVA-1 radiation on immunoglobulin production by activated B-lymphocytes.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteers’ EDTA blood by centrifugation using a Ficol–Hypaque gradient. The isolated cells were activated with CD40L, the control PBMCs were not. After 1 week the cells were irradiated with low doses UVA-1 radiation on 5 successive days. Immunoglobulin production in the presence of IL-10 was measured in the supernatants by ELISA. Results: UVA-1 irradiation of PBMCs resulted in a dose-dependent inhibition of immunoglobulin production by activated B-lymphocytes.

Discussion: The reduction of immunoglobulin production by activated B-lymphocytes in the dermal capillaries could be one of the possible explanations of the beneficial effects of low doses UVA-1 in SLE patients.

Prolonged susceptibility to local immunosuppression after repeated narrow-band UVB versus broadband UVB correlates with increased intraepidermal immunoglobulin production by activated B-lymphocytes

J.C. Ansel3, J.D. Glass2; United States.

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteers’ EDTA blood by centrifugation using a Ficol–Hypaque gradient. The isolated cells were activated with CD40L, the control PBMCs were not. After 1 week the cells were irradiated with low doses UVA-1 radiation on 5 successive days. Immunoglobulin production in the presence of IL-10 was measured in the supernatants by ELISA. Results: UVA-1 irradiation of PBMCs resulted in a dose-dependent inhibition of immunoglobulin production by activated B-lymphocytes.

Discussion: The reduction of immunoglobulin production by activated B-lymphocytes in the dermal capillaries could be one of the possible explanations of the beneficial effects of low doses UVA-1 in SLE patients.

Cis-urocanic acid stimulates NF-κB activation in primary human keratinocytes

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Ultraviolet radiation (UVR)-induced suppression of cutaneous cell-mediated immunity plays an important role in the development of photocarcinogenesis and susceptibility to infectious diseases. Photosensitization of urocanic acid (UCA) from its trans- to cis- isomer is an important event in the initiation of UVR-induced immunosuppression. The mechanism by which cis-UCA modulates immune responses is not fully defined. However, the expression of many genes involved in immunological and inflammatory responses are under the control of the transcription factor NF-κB, which is strongly upregulated by UVR. We therefore investigated the effect of UCA isomers on the activation of NF-κB in order to determine a possible involvement for this transcription factor in cis-UCA-mediated immunosuppression. Human primary keratinocyte cultures were established from biopsies of non-exposed buttock skin from healthy white-skinned volunteers (n=10). Keratinocytes were incubated in 100μg/ml of cis- or trans-UCA in PBS. Nuclear extracts were performed after 2 h and 24 h and analysed by electrophoretic mobility shift assay. cis- but not trans-UCA stimulated the release of NF-κB to the nucleus at both time-points and was greatest at 24h. The induction of immunosuppression by cis-UCA may involve the activation of the NFκB pathway in keratinocytes.

Transcriptional mutagenesis in prokaroytes and eukaryotes

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The majority of DNA damage-induced mutagenesis studies are based on systems involving DNA polymerase errors occurring in the vicinity of a lesion. Little information exists concerning the by-pass and miscoding events that may occur when RNA polymerase transcribes a damaged DNA template. We have conducted in vitro studies examining whether RNA polymerases generate mutant transcripts when they encounter DNA base lesions. These studies reveal that certain base deamination, oxidation and alkylation products are efficiently bypassed by RNA polymerases and are mutagenic at the level of transcription (transcriptional mutagenesis). Recent studies in our laboratory have addressed whether transcriptional mutagenesis can be detected and measured in bacterial and mammalian cells. We have utilized a transcriptional mutagenesis reporter system based on luciferase activity employing replication-defective constructs containing strong promoters into which we have engineered specific DNA damages at defined locations. We find that spontaneous base damage products such as uracil and oxidative base lesions cause transcriptional mutagenesis in both prokaryotic and eukaryotic cells. The extent to which this occurs for a given lesion and cell type is greatly influenced by the DNA repair background of the cell. These studies have important implications for the mechanisms leading to mutant proteins, particularly in non-dividing cells.
IL141
Mechanisms of UV-induced mutations
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In human skin cancers, more than 30% of all mutations in the p53 gene are transitions at dipyrimidines within the sequence context 5'-TCTG and 5'-CGG. Since CGs are methylated along the p53 gene, these mutations may be derived from UV-induced pyrimidine dimers forming at sequences that contain 5-methylcytosine. It has been shown that CPDs form preferentially at dipyrimidines containing 5-methylcytosine when UVB or sunlight is used for irradiation. In transgenic mouse mutation reporter genes, 24–32% of the solar light-induced mutations were at dipyrimidines that contain 5-methylcytosine and most of them were transitions. Our data make a strong case that CPDs forming preferentially at dipyrimidines with 5-methylcytosine are responsible for a considerable fraction of the mutations induced by sunlight in mammalian cells. Using photoproduct-specific photolyases transfected into cells containing mutational reporter genes, we demonstrated that CPDs (rather than 6-4-photoproducts or other UV-induced lesions) are responsible for the vast majority of UVB-induced mutations in mammalian cells. A major component of the mechanism that leads to CPD-induced mutations is the deamination of cytosine and 5-methylcytosine within the CPD prior to DNA replication. While mutations induced by UVB have been studied extensively, the mutagenic and carcinogenic effects of UVA irradiation have remained less clear.


IL145
Molecular mechanisms of UV-induced mutations in human cells proficient or deficient in DNA polymerase eta
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Cells from XP variant (XPV) are UV-hypersensitive because they are deficient in DNA polymerase η (Pol η), able to carry error free bypass of pyrimidine dimers. In the absence of pol η, other polymerases are able to bypass DNA lesions with lower fidelity. The high rate of misincorporation by these polymerases can explain the cancer-proneness of the XP variant patients. We have used UV-irradiated shuttle vectors to determine the mutagenic characteristics of DNA polymerases implicated in translesion synthesis in normal, XPV or XPV cells complemented with the wild type pol η gene. In normal cells, the bypass by pol η of UV-induced DNA lesions containing Cs appears to be more error-prone than those containing Ts. In XPV cells, the proportion of mutations at lesions containing Ts is increased, suggesting that pol η bypasses lesions containing Ts in a relatively error-free manner (2). UV-irradiation of host cells prior to transfection with unirradiated shuttle vectors increases the mutation frequency substantially in normal, XPV and corrected XPV cells to similar extent. This demonstrates that mutagenic polymerases are activated after UV-irradiation and produce mutations in undamaged DNA.


IL142
Potentiation of photodynamic therapy with hypericin by mitomycin C in the R1 mouse tumor model
F. De Gruijl
Hypericin, a polycyclic quinone obtained from plants of the genus Hypericum, has been shown to be a promising photosensitizer. We investigated in vivo the combination of hypericin-PDT and a bioreductive drug, mitomycin C. The R1 tumors were exposed to laser light (120 J/cm² at 595 nm) 24 h after an intravenous injection of hypericin (1 mg/kg). Hypericin-PDT alone significantly decreased tumor perfusion and oxygen tension as demonstrated by in vivo imaging techniques. Treatment with the combination of hypericin-PDT and mitomycin C significantly increased hypericin-PDT tumor cell kill. A lack of repair of non-transcribed DNA caused the observed synergism. The combination of hypericin-PDT and mitomycin C significantly increased tumor cell kill.

IL143
MAPK pathways and AP-1 transcription factors in DNA-damage responses
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Mitogen-activated protein kinases (MAPK) and AP-1 (Jun, Fos and ATF) transcription factors play key roles in regulation of cell proliferation, differentiation and apoptosis. Growth factors, cytokines and genotoxic agents can induce gene expression controlled by the dimeric AP-1 complex, the MAPK kinase family members JNK, p38 and ERK. In cells derived from patients with DNA repair syndromes (Ataxia Telangiectasia, Nijmegen Breakage syndrome, Xeroderma Pigmentosum, Cockayne’s syndrome) aberrant control of MAPK activity and AP-1-dependent transcription is observed. Recent results indicate that growth factors and DNA damaging agents like UV and MMC regulate AP-1-dependent gene expression differentially – and sometimes even antagonistically – via the stress-induced MAPK JNK and AP-1. By strongly inducing c-Jun phosphorylation via JNK, these genotoxic agents appear to differentially affect the activity of c-Jun/Fos and c-Jun/ATF2 dimers. This differential control of c-Jun/Fos and c-Jun/ATF2 activity by growth factors and genotoxic stresses may explain the opposite effects of these agents on cell cycle progression and cell death.

IL144
Experiments on the causal chain from uv-induced dna damage and repair to tumor formation
F. R. De Gruijl
Through all the intricacies of uv carcinogenesis have not been resolved, research has evolved to point where major steps have been identified. Central to the oncogenic process is the permanent dysfunction of cell cycle controlling pathways. UV radiation can contribute to such dysfunction through DNA damage and ensuing gene mutations. As substantiated by experiments with transgenic mice, the major defence mechanism appears to be nucleotide excision repair (NER), supplemented by cell death through apoptosis if extensive, residual or persistent damage hampers gene transcription and related vital cell functions. Cell cycle regulation appears to be linked to both of these defence mechanisms. A lack of repair of actively transcribed dna (as in xpa and csb mice) results in a strongly increased sensitivity to a) cell cycle arrest, which is associated with a low rate of dna replication upto 20 hours after uv exposure, and b) apoptosis (‘sunburn cells’). A lack of repair of non-transcribed dna (as in xpc mice) does not result in such an enhanced acute sensitivity, but results in a stagnant dna replication and an accumulation of tetraploid (4n) cells, peaking 72–96 hours after uv exposure. These events occur in absence of any overt apoptosis. Recent results with double knockout mice indicate that mismatch repair contributes to these late cell cycle effects in xpc mice. Earlier experiments showed that failure in ner greatly enhanced uv carcinogenesis: no repair in transcribed dna speeded up uv-induced tumor development 2 fold (in csb), no repair in non-transcribed dna 3 fold (in xpc) and an overall defective ner 4 fold (in xpa). The latter increase is possibly a moderating effect of apoptosis in xpa.

IL146
ALA-PDT dependence on up and down regulation of the PBGD gene in cancer cells
Z. Malis
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ALA PDT is highly dependent on the regulation and activity of the heme synthesis pathway in the target tumor. The regulation of porphyrin synthesis is dependent on porphobilinogen deaminase (PBGD), the third enzyme in the pathway. Traditionally, PBGD is known to act in the cytoplasm. We cloned the housekeeping and the erythroid PBGD gene and expressed them in glioma, melanoma and leukemic cells. Fluorescence immunostaining with anti-PBGD antibodies revealed a fraction of PBGD in the nucleus, which is associated with a low rate of DNA replication upt to 20 hours after UV exposure. Injection of a 2.5 mg/kg dose of mitomycin C 20 min before light application significantly decreased tumor cell survival and delayed tumor growth compared to PDT or mitomycin C alone. No more skin reaction was observed after the combination of mitomycin C and PDT than PDT alone. Our study demonstrates that combining hypericin-PDT with mitomycin C can be effective in enhancing tumor response with little side effects.
Combination regimens with ALA-PDT

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PDT as a cancer therapy has gained worldwide recognition. Similar to most other cancer treatments, PDT likely works best when combined with other therapeutic modalities. Two main strategies can be chosen, (1) the interaction of two modalities that work by independent mechanisms and, (2) the enhancement of those factors that compose PDT. Several pharmacological treatments are able to alter PDT effects by affecting the target cell prior to photosensitization. In most applications aminolevulinic acid (ALA)-dependent PDT is uniquely dependent on the cellular biology of the target cell, because its active metabolic contribution is required for optimal PDT effects. ALA offers additional versatility by the availability of its esters. Strategies that enhance the cellular capacity to form protoporphyrin IX are invariably associated with enhanced photosensitization. They also may enhance selectivity and the potential of fluorescence diagnosis. ALA-induced PpIX formation does not require rapid proliferation and may make this a promising approach for the treatment of more slowly proliferating cells, which are more resistant to conventional non-surgical therapies.

A new approach using porphyrins as radiosensitizing agents for solid tumors


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The biological effects of radiation affect both neoplastic and normal tissues. The nature and extent of such effects, however, depend on selected biological parameters (e.g., oxygen supply, cell cycle) and can be modified by chemical agents such as radiosensitizers, radioprotectors and chemotherapeutic agents. A precise control of the mode of action of the radiation is important in order to achieve the maximum effect on tumor tissue, while minimizing the effect on normal tissues. Most of the known and routinely used radiosensitizers are neither selective nor tumor specific. In this work we present a new selective and specific modality that increases the sensitivity of solid tumor tissue, especially of radio resistant, hypoxic tumor cells, to radiation. This modality is currently under early clinical evaluation and encompasses the application of Photofrin II, which is already used as a photosensitizer in photodynamic therapy (PDT) at predetermined times prior to irradiation.

Is PDT Generated Singlet Oxygen Luminescence a Predictor of Treatment Response in Vivo and in Vitro?

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Recently we demonstrated the feasibility of measuring singlet oxygen (1O2) luminescence at 1270 nm in cells in vitro and in rodents in vivo using a unique, near infrared sensitive photomultiplier tube. Since 1O2 is widely believed to be the major cytotoxic agent involved in PDT, this technique is of strong potential value as a dosimetry metric. In addition, time-resolved measurements allow calculation of 1O2 and photosensitizer triplet-state lifetimes under biological conditions. A sensitive detection system was set up to perform the 1O2 luminescence measurements during PDT treatments. Treatments were performed in vivo on AML5 leukemia cells in suspension sensitized with 1 mM ALA for 4 hours and irradiated with up to 45 J/cm2 of 523 nm light at varying irradiances. Viability was assessed using the flow cytometry propidium iodide and colony forming assays. Cell viability was found to correlate well with absolute cumulative 1O2 luminescence measured during treatment. In addition, PDT treatments were performed in vivo on the normal skin of nude mice using topical ALA and 523 nm treatment light. Biological response was evaluated daily for two weeks following PDT treatment using a skin redness score. We present preliminary results from this study.

Lysosome targeting by tetra-cationic porphyrins

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Several classes of cationic photosensitizers displaying appropriate structural features are known to localise inside the mitochondria driven by the transmembrane potential of the inner mitochondrial membrane. While the presence of the positive charge is essential for mitochondrial targeting, the effect of the lipophilic character of the molecule is not clear. Thus, we studied the efficiency of cell photoactivation and the intracellular localisation of derivatives of 5,10,15,20-tetrakis (4-N-methyl)-porphine whose degree of lipophilicity was varied through replacement of one methyl group with an alkyl chain of various length. HT 1080 human fibrosarcoma cells exposed to 0.25 µM porphyrin for 24 h and irradiated with increasing doses of red-light (0.45-27 J/cm2) were inactivated with a different efficiency. The efficiency of cell photoactivation increased with the increasing length of the hydrocarbon tail and lipophilicity and correlated with the efficiency of the porphyrin accumulation into the cells. Fluorescence microscopy studies demonstrated that these cationic porphyrins do localise rather selectively in acidic cellular compartment, presumably lysosomes, while mitochondrial localisation was not evident. In confirmation of this, studies on isolated mitochondria provide evidence that the dye uptake by these organelles is not modulated by the membrane potential but is exclusively dependent on the degree of lipophilicity.

Photosensitization with asymmetric chlorins: subcellular localization, efficacy and apoptosis induction in Jurkat cells

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An efficient sensitizer for photodynamic therapy (PDT) requires such photophysical properties like strong absorption in the infrared region and a high singlet oxygen quantum yield. Additionally, the photosensitization should induce apoptosis rather than necrosis. This death mechanism can be initiated when specific subcellular structures like cellular membranes, lysosomes or mitochondria are targeted. The main idea of this study is to test the effects of changes in the chemical structure of amphiphilic dihydroxychlorin in regards to intracellular localization and photosensitizing mechanism. Molecules where delivered to Jurkat cells in organic solvent or as liposomes preparation and their intracellular uptake, subcellular localization and photosensitizing efficacy were studied. The time for intracellular accumulation resulted to be strictly dependent on the molecule structure and the way of its delivery. All photosensitizers localize mainly in lysosomes; however, there is also an extra-lysosomal component, whit localization in mitochondria and Golgi apparatus. The ratio of necrosis vs. apoptosis resulted to be related to intracellular concentration and red light doses. These findings may have importance for future synthesis of photosensitizers.

Modulation of adhesion molecules expression in endothelial cells treated by photodynamic therapy

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Photodynamic therapy (PDT) leads to the production of reactive oxygen species (ROS) in treated tumours and associated vessels. These ROS can activate various cellular pathways leading to gene transcription and inflammation response. Here, we show that in endothelial cells (HMEC-1) photosensitization by pyropheophorbide-a methyl ester (PpMe) induced the activation of NF-kB. We have shown that NF-kB is functional since it binds to both ICAM-1 and VCAM-1 promoters and it induces their transcription. In contrast we were not able to detect the expression of ICAM-1 and VCAM-1 proteins in response to PDT, although we measured IL-6 secretion. Using specific inhibitors we showed that this non-expression of ICAM-1 and VCAM-1 is due to their degradation by lysosomal proteases. The photodynamic induced oxidation of the cellular membrane is important in this process since a treatment with BHA induces a slight expression of the two molecules after PDT. Moreover, we showed that PpMe photosensitization induces a transient downregulation of several integrins and plasma membrane proteins constitutively expressed. The absence of expression of ICAM-1 and VCAM-1 on PDT treated endothelial cells although the corresponding genes are transcribed, revealed that PDT could efficiently disturb the correct addressing of proteins to their target sites.
contain carotenoids

IL153

Energy transfer dynamics in biological and artificial antenna systems

T. Gillbr, F. Barker

IL154

Carotenoids and Sun Protection

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Photooxidative processes play a role in the pathobiocchemistry of several skin disorders. Upon exposure of skin to UV light, erythema is an initial biological response. Carotenoids as quenchers of singlet oxygen and scavengers of peroxyl radicals might serve in protection by dietary means. When β-carotene was ingested for 12 weeks, erythema formation (sunburn reaction) induced by a solar light simulator was significantly diminished from week 8 on (1). Similar protective effects were achieved with a diet rich in lycopene (2). In the group ingesting tomato paste, corresponding to a dose of 16 mg lycopene/day over 10 weeks, erythema formation was significantly lower than in the control group. Supplementation with a mixture of carotenoids (β-carotene, lycopene and lutein, 8mg/d each) protects against UV-induced erythema as efficiently as β-carotene alone (24 mg/d) (3). The extent of protection achieved with dietary carotenoids is not comparable to the use of sunscreen with a high sun protection factor. However, increasing the basal protection enhances the defense against UV light-mediated skin damage and may contribute to life-long protection.


IL155

Carotenoids protect human skin cells in vitro and primate eyes in vivo from damage by UV and near-visible radiations

T. Carlberg

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Carotenoids serve several important roles in photosynthesis. We have focused our work on their role as light-harvesting pigments in different photosynthetic antenna systems. Natural systems that will be discussed are the 850 complex of purple bacteria, the LHCI complex of green plants, the peridinin-chlorophyll complex of dinoflagellates. Artificial systems are e. g. carotenoporphyrins and caroteno (dicaroteno)-9-phthalocyanins. The main conclusions are that in many of these systems the energy transfer is on the sub-100 fs time scale and thus competes efficiently with the carotenoid S2 state relaxation time. The S1 state normally transfer energy more slowly and is generally less efficient. However, in order to achieve a high total efficiency transfer from both S1 and S2 is required in a stepwise process. In the artificial systems we are able to influence the energy transfer efficiency by modifying the energy level of the S1 state and the distance between the quenchers of reactive oxygen species, and singlet oxygen (1O2) in particular. Carotenoids as quenchers of singlet oxygen therefore offer some protection. They are also systems, do not quench chlorophyll triplet states although they can act as quenchers of singlet oxygen therefore offering some protection. They are also redox active, giving electrons to the highly oxidised chlorophyll known as P680 which normally oxidises water. This ‘side-reaction’ seems to be important in the photoprotection of PSII and also involves redox interactions with cytochrome b559 and chlorophylls bound within the reaction centre.

IL156

Human cell protection by carotenoids

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The combination of antioxidants (beta-carotene, vitamin E and C) provides strong in vitro protection of human fibroblasts against UVA (protection factor of approx. 5). The same antioxidants act synergistically in membrane protection of human lymphoid cells against nitrogen dioxide (radicals), with an in vivo protection factor of up to 10. Lycopene is able to protect with a factor of almost 1.8 under the same circumstances. It is suggested that the lycopene radicals (produced when lycopene reacts with a strong oxidizing radical such as nitrogen dioxide) are repaired by ascorbic acid as a result of the reorientation of the charged antioxidant radical inside the membrane. The damage of human fibroblasts due to photo activated protoporphyrin (in erythropoietic protoporphyrin) can also be considerably reduced by lycopene as well as the combination of beta-carotene, vitamin C and E. Against the background of promising results from the trials of the beneficial effects of lycopene in avoiding breast and prostate cancer and the synergistic benefits of combinations of antioxidants like lycopene and vitamin C or beta-carotene, vitamin E and C, provide, these combinations should be used to protect against specific damage of cells and tissue.

IL157

Role of β-carotene in the reaction centre of photosystem II

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he reaction centre of photosystem II (PSII) contains a heterodimer of two related proteins called D1 and D2 which bind all the cofactors involved in primary and secondary electron transfer leading to water oxidation and plastoquinone reduction. They also bind two β-carotene molecules which, unlike carotenes in other photosynthetic reaction centres and light harvesting systems, do not quench chlorophyll triplet states although they can act as quenchers of singlet oxygen therefore offering some protection. They are also redox active, giving electrons to the highly oxidised chlorophyll known as P680 which normally oxidises water. This ‘side-reaction’ seems to be important in the photoprotection of PSII and also involves redox interactions with cytochrome b559 and chlorophylls bound within the reaction centre.

IL158

Antioxidant activity of lycopene evaluated in topical application and in vitro

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Many substances with antioxidant activity are present in the human skin, and their concentrations are generally higher in the epidermis than in the dermis. Under the effect of an oxidative stress like that caused by UV rays, these substances are strongly depleted, especially in the external epidermal layer. This is the rationale for the topical use of antioxidants to protect the skin against solar radiation. We studied the protective activity of a product based on lycopene and a product containing a mixture of vitamins E and C. Photostimulation was applied with a solar simulator and the cutaneous response was evaluated instrumentally. Than we tested the antioxidant activity against UV radiation of the above substances in cell cultures of HaCaT. In vivo and in vitro experiments demonstrated that the lycopene-based product had a much greater protective ability than the product containing the mixture of vitamins. Therefore lycopene has suitable characteristics to be employed successfully in the prevention of cutaneous damage by free radicals.
Possible explanations will be discussed.

Chromatium minutissimum

Mixing, frequently used in mesocosm experiments, strongly influences the more than their prey, with eventual effects on the whole pelagic community. planktonic groups (notably bacterioplankton) when predators are affected. efficiencies may translate into positive feedbacks between enhanced UV and some planktonic ecosystems using large enclosures (mesocosms) set outdoors to rate in growth, survival, protein content, heterocyst frequency and fixation of carbon into carbohydrates, lipids, and proteins as an example. Changes in allocation patterns may vary with species and carry over to other trophic levels; thus information on partitioning is critical for modelling ecosystem dynamics under different UV scenarios. Using a polychromatic approach, we have developed BWFs for UV effects on allocation in Thalassiosira pseudonana (diatom), and Gymnodinium instriatum (dinoflagellate), and have found significant differences in UV sensitivity among pools and between species. In both species, sensitivity of allocation to lipids and carbohydrates is similar to overall photosynthesis, which differs between species. Of all pools, protein synthesis is the least sensitive, especially to UVA, though it is more sensitive to UVB. We conclude that carbon allocation is altered to conserve synthesis of nitrogen-rich compounds under ecologically relevant UV conditions. Comparisons will be made to the UV sensitivity of allocation patterns in natural phytoplankton populations under various nitrogen conditions.

FC159

Role of Carotenoids in B800-B850 Energy Transfer in the Light Harvesting Complex 2 (LH2) of Purple Bacteria

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Attempts to model the experimentally determined fast excitation energy transfer between the circular aggregates BCHI-B800 and BCHI-B850 of LH2 has been a hot topic for years. Despite recent progress, a detailed understanding is still lacking. In literature, one possible reason for these difficulties has been located in a special role of the carotenoids, bridging the space between the both BCHI compartments. Direct experimental approach to this question requires comparison of native LH2 with a corresponding carotenoid-depleted sample. Along this line, comparative investigations with native and carotenoid depleted samples on the influence of rhodopin (main carotenoid) on B800-B850 EET in LH2 of Chromatium minutissimum are reported. Contrary to theoretical predictions, no diminution of B800-B850 EET is observed in carotenoid-depleted LH2, but rather an enhancement. Possible explanations will be discussed.

FC164

A prospective placebo-controlled randomised double-blind study of tetra- caine gel for pain relief during topical photodynamic therapy

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Topical photodynamic therapy (PDT) is effective for superficial non-melanoma skin cancer (NMSC). The main limiting factor is pain during irradiation. Pain relief methods have not been assessed. We performed a prospective, randomised, double-blind, placebo-controlled study to evaluate the effect of tetracaine (Ametop)® gel for pain relief during and after topical ALA PDT. Patients with superficial basal cell carcinoma (n=27), Bowen's disease (n=13), or actinic keratosis (n=21) (lesions ≤2 cm diameter) were randomised to receive topical application of tetracaine (n=22) or vehicle (n=20) gel under occlusion for one hour pre-irradiation. Pain was assessed using a visual analogue scale (VAS) (0–10). Median VAS pain score during irradiation was 4 (range 1–7) amongst those allocated tetracaine gel, compared with 4.5 (range 1–10) for placebo (95% CI for difference in median VAS scores 0–3, p=0.08). There was no detectable difference in pain immediately after treat- ment (95% CI for difference in median VAS scores −2.5–1.0, p=0.63), or later. Thus, although there was a trend to lower pain scores during PDT for patients receiving tetracaine gel, this treatment was not significantly superior to placebo for pain relief during and after PDT for small lesions of superficial NMSC or dysplasia.

FC168

Effects of UV-B radiation on rice-field cyanobacteria

R. P. Singh, D. P. Hader

Institute for Botany and Pharmaceutical Biology, Erlangen, Germany. Cyanobacteria are the dominant microflora in rice-fields, contributing significantly to fertility as a natural biofertilizer. Recent studies reveal a continuous depletion of the stratospheric ozone layer, and the consequent increase in solar UV-B radiation reaching the Earth's surface. UV-B radiation causes reduction in growth, survival, protein content, heterocyst frequency and fixation of carbon and nitrogen in cyanobacteria. UV-B induced bleaching of photosynthetic pigments, disassembly of phylosomal complexes and alteration in membrane permeability have also been recorded in a number of cyanobacteria such as Anabaena sp., Nostoc sp. and Synechocystis sp. However, a variety of cyanobacteria produces photoprotective compounds such as water-soluble colorless mycosporine-like amino acids (MAAs) and the lipid-soluble yellow-brown colored sheath pigment, scytonemin, to counteract the damaging effects of UV-B. A circadian induction in the synthesis of MAAs by UV-B have been recorded in a number of cyanobacteria. Polychromatic action spectra for the induction of MAAs in Anabaena sp. and Nostoc commune also show the induction to be UV-B dependent peaking at 290 nm. Scytonemin, with an absorption maximum at 386 nm (also absorbs at 300, 278, 252 and 212 nm) was detected in many cyanobacteria. The role of these compounds in photoprotection of cyanobacteria will be discussed.
FC169
The Impact of UV Radiation on Planktonic Heterotrophic Flagellates and Their Bacterivory Rates
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Despite of the fact that several studies have revealed direct and indirect effects of solar UV radiation (UVR) on aquatic microbial communities, our understanding of its impact on the whole Carbon cycle is incomplete. Heterotrophic nanoflagellates (HNF) are the main consumers of picoplankton biomass in marine and freshwater ecosystems and consequently, they process a significant part of the carbon in the pelagic region. Information, however, on HNF species-specific sensitivity to UVR is limited. Furthermore, little information exists on the effect of UVR on HNF bacterivory rates. My presentation will address these two topics using examples from laboratory tests with HNF cultures and field experiments with natural HNF assemblages. Results from experiments with single species of HNF indicated that marine and freshwater representatives of the order Kinosteplastida were the most sensitive to UV radiation (e.g., DNA damage was 5 to 10 times higher than in chrysomonads and cryptomonads species), but also that cell targets were different among kinetoplastid species. In situ bacterivory experiments performed in a transparent alpine lake showed that UVR may significantly reduce (up to 25%) bacterial consumption in the water column.

FC170
Signalling pathways regulating the cell death and survival balance in response to Photodynamic therapy with hypericin
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Photodynamic therapy (PDT) has been described as a promising modality for the treatment of cancer. PDT involves the combination of a photosensitizer, oxygen and light to induce death of cancer cells by the photochemical generation of reactive oxygen species (ROS). We have considered the impact of hypericin, a naturally occurring photosensitizer isolated from St. John’s wart (Hypericum perforatum) with promising activities in clinical PDT, as an inducer of apoptosis in tumor cells both in vitro and as well in vivo. Our recent data show that apoptosis is a major tumor cell response to hypericin-mediated PDT. Photosensitization of hypericin, which localizes in cytosolic membranes of the endoplasmic reticulum and golgi apparatus, results in rapid cellular calcium overload, disruption of the mitochondrial membrane potential along with the release of cytochrome c from mitochondria and activation of the apoptosome, leading to cell death. Concurrently, the mitochondria-mediated caspase activation cascade, photosensitized hypericin induces the sustained activation of the p38 MAPK signal, which is functionally involved in the up-regulation of cyclooxygenase-2 and in a protective response against the oxidative stress imparted by PDT. The current knowledge on the signalling pathways regulating the cell death/survival balance following hypericin-mediated PDT, will be discussed.

FC171
Physical properties of membranes affect the uptake, topography and efficiency of photosensitizers
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Hydrophobic sensitizers partition mainly into lipid membrane environments. Singlet oxygen diffuses rapidly out of the membrane and decays very rapidly. Membrane-localized damage can be caused only when singlet oxygen diffuses inside the membrane. Deeper insertion of sensitizer increases the dwell time of 102 in the membrane and enhances sensitizing efficiency. We synthesized photo- and hematoporphyrins with varying lengths of alkyl carboxylates, which anchor them at the water interface. Relative vertical depths were determined by iodide fluorescence quenching. Absolute depths were evaluated by the parallax method. A strong depth-dependent effect on the sensitization efficiency, measured with singlet-oxygen traps, was observed, even when the depth changed by a few Angstroms. Membrane additives, such as DMPC or cholesterol, pushed the porphyrins deeper in the membrane. Stronger than changes in efficiency as a result of change in temperature, phase transition or fluidity were observed. The vectorial insertion raises the question whether its extent can be predicted by theoretical or experimental partitioning measurements. A study of tens of porphyrins, modified chemically, revealed that when possessing similar structures, the liposomes’ binding constants correlate with the lipophilicity parameter logD. These results highlight the possible use and limitations of lipophilicity parameters for the prediction of membrane binding.

FC172
Fluorescence lifetime imaging and spectral behaviour of Protoporphyrin IX (PpIX) in human tumor cells – correlation with photodynamic inactivation
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Fluorescence lifetime imaging: fluorescence spectroscopy and photobleaching experiments were performed to characterise PpIX in different human cancer cell lines (glioblastoma, ovarian carcinoma and breast cancer). Lifetimes measured with a picosecond laser diode and a time-resolving image intensifying camera system were correlated with photodynamic inactivation. Before irradiation, fluorescence lifetimes between 11 ns and 24 ns were mainly measured. During and after irradiation, a pronounced decrease of fluorescence lifetimes down to 5-7 ns was measured, which in part could be correlated with the formation of photoproducts. Furthermore, short fluorescence lifetimes around 1.5 ns were attributed to PpIX aggregates. In addition, photobleaching as well as photofotoproduction formation was closely correlated with the initial fluorescence intensity and therefore the intracellular PpIX amount. For cells with high intracellular amounts, photobleaching was rapid. The formation of photoproducts, characterised by additional fluorescence maxima, became obvious. Photodynamic inactivation seemed to decrease with increasing intracellular sensitizer amount, due to the increasing formation of photoproducts and aggregates, and was most pronounced in T47-D cells. In conclusion, fluorescence lifetime imaging is a valuable tool to obtain additional data on intracellular distribution, aggregation and photoproduction formation.

IL170
Signalling pathways regulating the cell death and survival balance in response to Photodynamic therapy with hypericin
P. Agarapina
Catholic University of Leuven, Leuven, Belgium.
Photodynamic therapy (PDT) has been described as a promising modality for the treatment of cancer. PDT involves the combination of a photosensitizer, oxygen and light to induce death of cancer cells by the photochemical generation of reactive oxygen species (ROS). We have considered the impact of hypericin, a naturally occurring photosensitizer isolated from St. John’s wort (Hypericum perforatum) with promising activities in clinical PDT, as an inducer of apoptosis in tumor cells both in vitro as well as in vivo. Our recent data show that apoptosis is a major tumor cell response to hypericin-mediated PDT. Photosensitization of hypericin, which localizes in cytosolic membranes of the endoplasmic reticulum and golgi apparatus, results in rapid cellular calcium overload, disruption of the mitochondrial membrane potential along with the release of cytochrome c from mitochondria and activation of the apoptosome, leading to cell death. Concurrently, the mitochondria-mediated caspase activation cascade, photosensitized hypericin induces the sustained activation of the p38 MAPK signal, which is functionally involved in the up-regulation of cyclooxygenase-2 and in a protective response against the oxidative stress imparted by PDT. The current knowledge on the signalling pathways regulating the cell death/survival balance following hypericin-mediated PDT, will be discussed.

FC173
Relationship between mTHPC photobleaching and cell viability during in vitro photodynamic therapy
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Explicit dosimetry in photodynamic therapy (PDT) is difficult due to the complex and dynamic interactions between the sensitizing drug, oxygen, and the treatment light. An alternative model is based on the assumption that photobleaching and cellular damage are both dependent on interactions with singlet oxygen. As a result, a decrease in fluorescence could be used as a predictor of biological damage if the correlation between the two were known. To test this hypothesis, HT29 and MatLyLu cell lines were sensitized with mTHPC and treated with 650 nm light. Cell samples were withdrawn during treatment and cell viability was assessed using colony formation. Photopsensitizer fluorescence was monitored during treatment. The relationship between relative photobleacher photobleaching and cell viability was determined for a range of fluence rates and sensitizer incubation concentrations. The relationship was independent of fluence rate, but dependent on incubation concentration, where high sensitizer concentrations required less photobleaching to achieve equivalent survival fractions. This suggests that the in vitro biological response to PDT damage may not be a simple function of singlet oxygen generation.

FC174
Effects of Polysaturated fatty acids on 5-aminolevulinic acid based photo sensitisation in four different human cancer cell lines
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Norwegian University of Science and Technology, Trondheim, Norway.
The purpose of the study was to examine whether n-6 and n-3 fatty acids (PUFAs) could potentiate the effect of photodynamic therapy (PDT) in human cancer cell lines. The effects of 5-aminolevulinic acid (5-ALA)-PDT with/without pretreatment with docosahexaenoic acid (DHA) or arachidonic acid (AA) were tested in A-427 (lung adenocarcinoma), A-172 (glioblastoma), SW-480 (colon carcinoma) and WiDr (colon carcinoma) cells. The effect of 5-ALA-PDT, using a PUVA unit, varied between different cell lines. Pretreatment with DHA/AA (10μM, 48 h) did not influence the cytotoxic effect of 5-ALA (2 mm), except in A-427 cells. The difference in endogenous PpIX production between the cell lines increased during incubation of 5-ALA. The highest PpIX production efficiency in WiDr cells (400μmol/mg protein) and was four times that in SW-480 cells. Pretreatment with α-tocopherol (50μM, 48 h) protected against 5-ALA-PDT induced cytotoxicity in all cell lines. The level of malondialdehyde (MDA), a secondary lipid peroxidation product, was not affected by 5-ALA-PDT alone, but a several-fold increase was observed in all cell lines treated with DHA/AA and 5-ALA-PDT. Results indicate that endogenous PpIX production is cell line dependent, and that lipid peroxidation together with other mechanisms are involved in cell death after 5-ALA-PDT.
Photodynamic therapy (PDT) mediated cell cycle deregulation and apoptosis
incubation with IC50 concentration of sensitiser and illumination with 3jcm^2
Propyl and n-Pentyl derivatives, both derivatives have previously been associ-accumulation of cells in S-phase, in addition to the necrotic cell death which
tained at 24 hours post treatment. In contrast MB produced a prolonged
FITC/PI assays was completed at 1 or 24 hours post PDT. The MB derivatives
PDT, flow cytometric analysis of both DNA cell cycle and dual Annexin-V-
ality, coupled with an improved phototoxicity: dark toxicity ratio compared to
using a 664nm laser. The derivatives demonstrated enhanced cell killing abil-
FC175
Photodegradation and phototransformation of 5,10,15,20-tetrakis(m-
hydroxyphenyl)bacteriochlorin
(m-THPC) in solution
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Nancy, France, 2Institute For Cancer Research, The Norwegian Radium Hospital, Oslo, Norway.
In the present study we investigated some processes involved in the pho-
todegradation of the bacteriochlorin m-THPC, and compared these process-
ents of the photosensitizer m-THPC. The stock solution of m-THPC was diluted in pure methanol or in Phosphate Buffer Solution (PBS) supplement-
ed with Human Serum Albumin (HSA). Photobleaching measurements were
carried out by spectrophotometry and spectrorfluorimetry. The m-THPC pho-
tobleaching rate follows a fast first order kinetics, unlike those of m-TPHC which
follows a slower second order kinetics. Photobleaching of the aggre-
gated form leads to transformation of 50% of m-THPC into m-THPC (photo-
transformation) while only 5% of the HSA bound m-THPC gives rise to m-
THPC upon irradiation (true photobleaching). Under illumination, the HSA
bound bacteriochlorin can generate singlet oxygen and possibly also radicals,
which can oxidize m-THPC molecules and degrade the tetrapyrrolic ring into
small fragments with no absorption in the visible region. While in the case of
aggregate cytometrically, MB can be利好fow singlet oxygen to penetrate into
the aggregates and react, thus m-THPC molecules may act as oxidizers for
other m-THPC molecule. The fact that m-THPC is quite photolabile may be
an advantage for PDT, since the bleeding may limit the photodamage to
ormal tissues.
FC176
POT effects on the adhesive properties of cultured human carcinoma and
glioma cells
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1Rostov State University, Rostov-on-Don, Russian Federation, 2Institute for Cancer Research, Oslo, Norway.
We studied PDT effect on the adhesive properties of cultured WiDr carcinoma
and DS45m glioma cells. Sublethal phototranssisation with TPPm, Mitotracker Red (MTR), or ALA caused inhibition of trypsin-induced cell detachment. In order to reveal the cellular targets for PDT, we studied the intracellular localisation of the sensitisers. TPPm was selectively localised in the plasma membrane, whereas MTR was found in mitochondria. Both gran-
ular and diffuse fluorescence of ALA-derived protoporphyrin IX (PpIX) was
observed in the perinuclear cytoplasm but not in the plasma membrane of
WiDr cells stained 2 h with 1 mM ALA, whereas some membrane staining was
found in glioma cells. Spectral measurements showed accumulation of PpIX in
WiDr. An efflux PpIX into the medium was observed after 1-h incubation.
This efflux might be responsible for photompaired cell detachment. On the other hand, ALA-PDT induced inhibition of cell detachment at shorter incubation (15 min), when newly synthesized PpIX should still be in mito-
ochondria, indicated that not only the plasma membrane, but also the mito-
ochondria, remote from the cell surface where adhesion occurs, may partici-
olate in ALA PDT impairment of trypsinization efficiency as well as it was
observed for MTR phototranssisation.
FC177
How do methylene blue derivatives kill V79 cells during photodynamic
therapy?
R. K. Tyler, S. L. Hankin, S. A. Gorman, D. J. Vernon, J. Griffiths, S. B. Brown;
University of Leeds, Leeds, United Kingdom.
Photodynamic therapy (POT) mediated cell cycle deregulation and apoptosis
were characterised using Methylene Blue (MB) derivatives in V79 chinese hamster lung fibroblast cells. Nuclear localising MB was compared to the n-
Propyl and n-Pentyl derivatives, both derivatives have previously been associ-
ated with mitochondrial localisation. All POT PDT experiments involved a 2 hour incubation with IC50 concentration of sensitiser and illumination with 3jcm^2
using a 664nm laser. The derivatives demonstrated enhanced cell killing abil-
ity, coupled with an improved phototoxicity: dark toxicity ratio compared to
MB, as determined by the MTT cell viability assay. To further address the rela-
ship between photosensitisrer localisation and the cellular response to POT, flow cytometric analysis of both DNA cell cycle and dual Annexin-V-
FITC/PI assays was completed at 1 or 24 hours post PDT. The MB derivatives
induced a rapid, significant amount of apoptotic cell death, accompanied by
an accumulation of cells in S-phase of the cell cycle. This effect was not sus-
tained at 24 hours post treatment. In contrast MB produced a prolonged
accumulation of cells in S-phase, in addition to the necrotic cell death which
was observed. This data will improve the understanding of the mechanism of
POT-mediated responses in in vitro model systems.
FC178
Development of a test system for mutagenicity of photosensitizers using
Drosophila melanogaster
T. G. Sm1, H. I. Schultmaker2, M. J. Nivard2;
1University Medical Center, Leiden, Netherlands, 2Leiden University Medical Centre (PhotoBioChem Leiden NV), Leiden, Netherlands, 3Leiden
University Medical Centre (Toxicogenetics), Leiden, Netherlands.
In the Ames test white light itself was proven to be mutagenic and the effect
influenced by the light source. To develop a robust photo-mutagenicity test
we studied Drosophila melanogaster as a test organism. Drosophila was cho-
osen because of the existence of the Somatic Mutation and Recombination
Test (SMART), a genotoxicity test that studies homologous recombination.
Based on the SMART we introduced the factor light and optimised the test
conditions with the introduction of Methylene Blue (MB) as positive control for
photomutagenicity. In order to achieve this we determined the light and
sensitizer concentration in a range where mutagenicity could be established
and light dependent toxicity occurred as well (50%). We demonstrated that
MB was a good positive control in the modified SMART. This drug showed a
positive result in the presence of broadband white light and a negative result
under Sylenes B and DP mme and HP are under current investigation with this
photo-SMART. We conclude that MB serves as a good positive con-
trol for the developed photo-SMART. This test system offers the possibility to
detect photogenotoxicity not only of compounds that form stable photopro-
ducts upon illumination but also compounds that form short-lived photo-products.
FC179
Complexes of adenovirus with polycations increase the efficiency of photo-
cytologically mediated transduction
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Poor efficiency of adenoviral gene transfer to target cells is a major limitation
of adenoviral gene therapy. Inefficient gene transfer occurs in the absence of
cocssackie- and adenovirus receptor (CAR) on the cell surface, and can be over-
come by enhancing viral entry with cationic molecules. Such cationic
molecules with recombinant adenovirus imply a loss of transduction
specificity. Therefore, we have investigated the potential of a novel site spe-
cific (i.e. light specific) photochemical treatment, named photochemical
internalization (PCI), to enhance gene delivery of adenovirus serotype 5 (Ad5)
complexed with the polycationic polymers poly-L-lysine (PLL) and SuperFect™.
Cell lines differing in their receptiveness to Ad5 were infected with amounts
of virus transducing about 2% of the cell population by conventional aden-
ovirus infection. The combination of polycations and PCI enabled a substan-
tial increase in reporter gene expression, up to 75% of the cells were positive
after the combined treatment, and the effect was most prominent in cell lines
expressing moderate to low levels of CAR. Thus, we conclude that the PCI
treatment with Ad/polycationic complexes as vector present an opportunity
to obtain high cell infectivity levels with low viral doses.
FC180
Tissue detection of diphenylchlorin sensitizer (SIM01) by fluorescence
and high-performance liquid chromatography
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Tissue distribution and elimination kinetics of the photosensitizer 2,3-dihy-
dro-5,15-di(3, 5 dihydroxyphenyl)porphyrin (SIM01) were analysed in biolog-
cal samples from mice tissues by OFS and HPLC. Measurements were per-
formed 4, 6, 12, 24 and 48 h after an intraperitoneal injection for SIM01 doses
of 2, 5 and 15 mg/Kg. With OFS the evolution of the fluorescence peak was
analysed at 640 nm. HPLC measurements were performed on a RP-18 column (100 x 4.6 mm) with a multilinear gradient of aqueous phos-
phate buffer (40 mM NaH2PO4) and organic phase (125 mM tetrabutylam-
monium dihydrogen phosphate in Methanol) at 400 nm. The fluorescence
in the elimination organs was dose-dependent and varied according to the
interval after injection. Elimination seemed to concern essentially gall-
bladder and liver and stool, whereas maximum fluorescence reached respectively
20,000 ; 2,800 and 15,000 cps for 5 mg/Kg, 6 h after injection. Among the tis-
ues examined with HPLC, the highest SIM01 levels were found in stool, urine,
liver, gallbladder and pancreas. Liver, gallbladder, and stool homogenates
from drug-treated animals contained an additional peak detectable only
after injection of 15 mg/Kg. Our HPLC determinations and in vivo fluores-
cence detection of SIM01 gave comparable kinetic profiles. These techniques
should be considered as complementary rather than exclusive.
FC181 Photodynamic therapy and fluorescent diagnostics of breast cancer with photosensone
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1Cancer Research Center AMS of Russia, Moscow, Russian Federation; 2Organic Intermediates and Dye Institute, Moscow, Russian Federation.

PDT and FD using Photosensone (Phthalocyanine Al,NOIPIC, (PS) in dose 0.5 mg/kg of body weight) were provided in 131 patients with breast cancer. In 21 patients with T1-T3NO-LW0 preoperative PDT of primary tumor was done, radical mastectomy was performed with the accessory excision of 7 days after PDT history. No recurrences had 110 patients after breast cancer combined treatment. FD of tumor borders, accumulation of PS in tumor, skin before and during PDT was fulfilled. We used semiconductor laser, λ = 672 ± 2 nm for PDT. Intertitial irradiation has been done in light dose 150 J/cm² for primary tumor. In patients with skin metastases multiple surface irradiations were done in light dose 100 J/cm² and total light dose 300–800 J/cm².

Pathomorphosis of different degree has been found in 13 cases. In recurrences of breast cancer we’ve had ORR of 86.9% with complete response (CR) in 51.2%. In a year after PDT in cases of CR we had CR in 36.6%, local recurrence in 23.1%, distant metastasis in 40.4%. The best results were got in 39 patients with solitary metastases – 91.2% CR. Our experience show pronounced efficacy of PDT for breast cancer.

FC182 Synergism of ALA-PDT and methotrexate
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Wellman Laboratories of Photomedicine, Boston, MA, United States.

E. have evaluated the combination of methotrexate (MTX) with PDT using aminolevulinic acid-induced protoporphyrin IX in prostate cancer cells. LNCaP cells were exposed to 1mg/L MTX for 72 hours. MTX was removed, cells were incubated with ALA and irradiated after 4 hours. Survival was assessed by MTI and clonogenic assays. MTX treatment of LNCaP cells resulted in growth arrest and apoptosis. Subsequent exposure to ALA for 4 hours resulted in more than fourfold PixI formation. Lethal cytotoxicity was consequently enhanced. Colony formation as a measure of long-term efficacy was also reduced with MTX pretreatment. The enhancement was statistically highly significant and synergetic. The interaction of MTX and ALA-dependent PDT was sequence dependent. When LNCaP cells were first exposed to MTX and subsequently for 72 hours to MTX, there was no reduction of long-term survival. This indicates that the enhancement of cytotoxicity by MTX in the combined regime is due to the increase in ALA-induced PixI formation. The combination of short-term chemotherapy followed by ALA-PDT may help reduce dose-related toxicity of either component and thus improve existing PDT regimens for cancer treatment.

II183 Putting In Vitro Phototoxicity Testing into Practice: The Impact of Comments from OECD Member Countries on a Scientifically Validated Method
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Font face error! It is often criticised that international commenting on newly developed test methods does not always contribute to increasing their credibility. In contrast, if a new methodology has become highly defined as the result of test optimisation during a validation trial, any change made based on the comments can bear the risk of reducing the test performance. Although well-developed methods should be robust to slight modifications each method has crucial steps that cannot be modified. If the nature of comments is likely to put significant risk on a test method the most promising solution is to have face-to-face meetings of those involved in validating a method and those representing the most substantial critics on the method. This has been successfully practised to support adoption of the new OECD test guideline 432 “in vitro 313 nrn phototoxity test”. The discussions between experts resulted in a carefully revised test method description, increasing the flexibility wherever possible, and keeping it strict wherever necessary. In addition, to address concerns about the prediction model used, a retrospective biostatistical analysis was performed on all data available from the validation studies that resulted in an improvement of both the prediction model and the software used to predict phototoxic potential.

II184 Photosensitization of thymine nucleobase by benzophenone derivatives as models for photoinduced DNA damage
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Much attention has been devoted to the phototoxicity and chemistry of DNA and its constituents, with special emphasis on triplet-mediated photodynamic dimerization. However, the mechanistic aspects of ketone photosensitization of thymidine still require further investigation. Thus, although efficient quenching of triplet benzophenone by thymidine was observed \( k = 1.4 \times 10^7 \text{M}^{-1} \text{s}^{-1} \), this interaction has been explained either as an electron transfer or as an energy transfer mechanism. Both processes are energetically disfavored in the isomerized nucleoside, although they could be feasible inside DNA, due to the lower ionization potential and triplet energy of the thymine base in the biopolymer.

In the present work, the photoinduced intermolecular processes taking place between thymidine and the excited triplet states of benzophenone derivatives will be elucidated. The anti-inflammatory ketoprofen and the hypolipidemic drug fenofibric acid have been reported to promote DNA damage by oxidative single strand break and formation of cyclobutane thymine dimers. The present photophysical and photochemical study includes these two drugs, as well as two synthetic dyads with the ketoprofen sensitizer directly attached to the thymidine nucleoside at two different sugar positions. The main process observed in these systems is formation of oxetane adducts, rather than electron or energy transfer.

II185 Some aspects of the photosensitization induced by antibacterial fluoroquinolones in native and cell DNA
G. De Guidi;
University of Catania, Catania, Italy.

In the complex field of the phototoxicity induced by antibacterial fluoroquinolones, Rufloxacin (RFX) and Ofloxacin (OFX) are characterized by a particular pattern of photodegradation with a negligible quantum yield of defluorination and a N-demethylation under aerobic conditions. For both compounds the photoinduced production of 8-hydroxy-2'-deoxyguanosine (8-OH-dGuo), a bio-marker for aging and carcinogenesis in DNA, is mainly associated with a type II mechanism. This is in agreement with the fact that the drug photoreactive state is a triplet, able to transfer with high efficiency its energy to molecular oxygen, giving rise to singlet oxygen. Moreover, the RFX photosensitized formation of strand breaks in DNA of human non immortalized fibroblasts, determined via Comet assay measurements, is correlated with the intracellular content of 8-OH-dGuo. This data should better clarify the relationships between the ability of fluoroquinolones to produce oxygen activated species and their phototoxicity in vitro. In addition, the role of metal ions at very low concentrations is taken into account, as a valid tool in inhibiting toxic effects photoinduced by fluoroquinolones, as well as a potential strategy for modulation the photochemical pathways involved in the photodegradation of these molecules.

II186 Photoseparated reactions of isolated and cellular DNA
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Increasing attention is devoted to the determination of molecular effects of endogenous photosensitzers and photoexcited drugs on nucleic acids. Most of the photosensitized reactions are oxygen dependent as the result of the so-called photodynamic action. A large body of information is now available on the decomposition pathways of DNA that involve type I (one-electron oxidation) and type II (singlet oxygen oxidation) photosensitization mechanisms. Thus guanine is the main target of the latter photodamage reactions that give rise predominantly to 8-oxo-7,8-dihydroguanine (8-oxoGuA) in both isolated and cellular DNA. In addition 8-oxoGuA may also be generated by OH radical that is produced by the Fenton reaction from the unreactive superoxide radical, a side-product of type I mechanism. This was shown to be case to explain some of the phototoxic effects of UVA radiation on cellular DNA. However, the main DNA oxidation pathway giving rise to 8-oxoGuA was found to involve singlet oxygen that arises from still unknown photosensitizer. Photoexcited fluoroquinolones were also found to act on cellular DNA by singlet oxygen and radical oxidations. However, and this was also found for UVA radiation, an energy transfer mechanism that mostly leads to the formation of thymine containing cyclobutadiene derivatives is operative for enoxacin.

70
IL187
Fluroquinolone photosensitised skin tumorgenesis
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The fluoroquinolones (FQ) are systemic antibiotics used against a broad spectrum of bacteria, including B. anthracis. With recent large scale prophylactic use of these drugs it is important to understand their photochemical, phototoxic and particularly, their phototumorigenmic properties. Lomefloxacin (LOM) photosensitises human skin to solar UVA radiation (315–400 nm) and we have previously shown that LOM is a potent phototumorigen; a single systemic treatment with 0.1mg LOM is phototumorigenic in mouse skin irradiated with 20 J/cm² UVA. Many LOM photosensitised tumours contained C-T or CC-TT mutations in p53 which are indicative of thymine dimmer (T²T²) formation normally associated with UVB (280-315nm) exposure. Differing techniques have demonstrated that LOM is capable of photosensitizing T²T² in human skin cells in vitro and we have recently shown that T²T² are also formed in mouse skin after systemic LOM and UVA treatment. Although LOM photosensitised T²T² are highly mutagenic and are likely to be important in tumour initiation, the mechanisms by which they are formed are unclear. Data on the properties of the LOM triplet suggest that both its lifetime and energy are unlikely to favour a triplet-triplet energy transfer mechanism. This work was funded in part by a European Union 'Environment and Health' grant.

IL190
Selenium augments immunity and resistance to oxidative stress – a role in photoprotection?
R. C. McKenzie
University of Edinburgh, Edinburgh, United Kingdom.

Selenium (Se) is an essential trace mineral obtained from the diet and is incorporated into selenoproteins, through which many of its protective effects are mediated. Selenoproteins include the antioxidant enzyme families glutathione peroxidases (GPx) and thioredoxin reductases (Tr). These proteins detoxify reactive oxygen species. Se deficiency suppresses the effectiveness of both humoral and cell-mediated immunity – which is of concern since human Se intake is below dietary recommendations in many European countries. Moreover, dietary Se supplements have been shown to decrease the incidence of certain types of cancers in humans and to protect mice against ultraviolet radiation (UVR)-induced skin cancers. Dietary Se supplements also protect human skin against sunburn cell formation and raise the minimal erythmal dose. The goal of my colleagues and I has been to determine mechanisms by which Se protects skin against UV-mediated damage. From these studies we show that Se protects keratinocytes against UVR-induced oxidative DNA damage, UVR-induced lipid peroxidation and the induction of immunosuppressive cytokines such as IL-10 and tumour necrosis factor alpha. In mice, dietary Se intake appears to regulate Langerhans cell numbers and lymphocyte responsiveness. While Se does not act as a sunscreen, Se compounds show promise as a photoprotectant against UVR-mediated skin damage.

IL192
Vitamin E reduces the lipid peroxidation associated with EPA supplementation and enhances resistance to UVR-induced erythema in human skin
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1University of Manchester, Manchester, United Kingdom; 2Leiden University, Leiden, Netherlands.

Dietary w-3 PUFA's are photoprotective, despite their susceptibility to UVR-induced oxidative degeneration. Eicosapentaenoic acid (EPA), 4g daily for 3 months, conferred UVB protection, whilst causing a mild increase in oxidative stress. We examine whether additional vitamin E influences the effect of EPA on UVB-induced erythema and epidermal oxidative damage. A lower EPA dose is utilised, which may be more suitable for long-term supplementation. Initially, we assessed effects of vitamin E alone (400 IU/day for 8 weeks, n = 8); MED to UVB was unchanged and non-significant reductions occurred in skin levels of malondialdehyde (MDA), an indicator of lipid peroxidation. Next, 16 volunteers took 2g EPA + 300 IU vitamin E (n = 8) or 2g EPA alone (n = 8), daily for 8 weeks. MED to UVB increased in the EPA+E group (p < 0.01), but was unchanged following EPA. Supplements increased epidermal EPA by 80% (p < 0.005), but did not influence MDA in unirradiated skin. UVB (3x MED) did not alter vitamin E and MDA levels in the EPA+E group, but in the EPA group vitamin E fell to 50% and MDA increased to 183% of values in the dual supplement group (p < 0.005). Additional vitamin E helps maintain epidermal antioxidant defences, optimising the beneficial effect of EPA.

IL193
Pro-carcinogenic Activity Of Beta-carotene, A Putative Systemic Photoprotectant
H. S. Black
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Beta-carotene is a strong singlet oxygen quencher and antioxidant. Epidemiological studies have implied that an above average intake of the carotenoid might reduce cancer risks. Earlier studies found that the carotenoid, when added to commercial closed-formula rodent diets, provided significant photoprotection to UV-carcinogenesis. Clinical trials found that beta-carotene supplementation evoked no change in incidence of non-melanoma skin cancer and that smokers suffered a significant increase in lung cancer incidence. Recently, employing a carotenoid supplemented semi-defined diet, not only was no photoprotective effect found, but significant exacerbation of UV c-carcinogenesis resulted. A mechanism was proposed by which vitamin C repaired the carotenoid radical cation, a strongly oxidizing radical resulting from interactions with oxidizing species and which, if unpaired, might contribute to the exacerbating effect on UV-carcinogenesis. However, when hairless mice were fed beta-carotene supplemented semi-defined diets with varying levels of alpha-tocopherol and vitamin C, neither significantly ameliorated the exacerbation of UV-carcinogenesis by the carotenoid. It was concluded that the non-injurious or protective effect of beta-carotene found in the closed-formula rations might depend on interaction with other dietary factors that are absent in the semi-defined diet. At present, beta-carotene use as a dietary supplement for photoprotection should be approached cautiously.
IL194  Heat shock proteins and potential hazards of extrinsic photoprotection
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University of Vienna, Austria.
Heat shock proteins (hsp) are a group of diverse proteins expressed in
response to various types of stress. The function of most hsps can be
described as “molecular chaperones” indicating their ability to prevent denat-
uration of proteins and to help in refolding or degradation during stress.
There are several lines of evidence that pretreatment with hyperthermia pro-
tects human keratinocytes from UV-induced cell death, reducing the forma-
tion of sunburn cells. It is assumed that hsps, particularly hsp72, are involved in
this effect. Although at first view the inhibitory effect of hyperthermia on
sunburn cell formation might be interpreted as beneficial (leading to less UV-
induced cell death in the skin), it might be harmful if it would allow the sur-
vival of cells with DNA lesions that could eventually initiate mutagenesis and
carcinogenesis. Furthermore, the expression of hsp72 and the response to heat
stress decreases in aging skin which has been linked to the pathophysiology of
photoaging. Therefore, hsps appear to be an interesting pharmacological
target with regard to photodamage of skin. Hence non-toxic hsp induc-
ers are being developed although their long term safety is not yet warranted.

IL197  Singlet oxygen and the activation of a genetically controlled suicide pro-
gram in Arabidopsis thaliana
K. Appel, C. Laloi, A. Danon;
ETH Zürich, CH 8092 Zürich, Switzerland.
The conditional flu mutant of Arabidopsis thaliana makes it possible to
induce the release of singlet oxygen in a controlled and noninvasive manner.
The flu mutant accumulates protochlorophyllide in the dark. Following a
dark-to-light shift singlet oxygen is produced. Generation of singlet oxygen
starts within the first minute of illumination and was shown to be confined
to plastids. Immediately after the shift mature plants stopped growing, while
seedlings bleached and died. These responses do not result merely from
physicochemical damage. Instead they reflect the activation of distinct stress
response programs, as indicated by the recovery of more than 30 seed-site
suppressors mutations that abrogate the reaction. Two distinct signal trans-
duction pathways were defined, which diverged from a single genetic locus,
Terminator1, that is indispensable for the processing and transmission of the
initial stress signal.

IL198  Inducible RNAi of chlorophyll biosynthetic genes in transgenic tobacco
plants
F. Börnke, U. Sonnewald, S. Chen;
Institut für Pflanzen gentechnik und Kulturpflanzenforschung, Gatersleben,
Germany.
Down regulation of endogenous genes via post-transcriptional gene silenc-
ing (PTGS) is a key to the characterization of gene function in plants. However,
strong constitutive silencing often leads to pleiotropic effects which make it
difficult to directly relate phenotype to gene function. Here, we show that
efficient and strong genetic interference can be achieved in a chemically
inducible fashion allowing for temporal and spatial control of gene silencing
in transgenic plants. To this end, transgenic tobacco plants were established
expressing dsRNA in the form of intron-spliced hairpin structures under the
control of the ethanol-inducible alc gene expression system. Targeting mag-
nesium-chelatase subunit 1 (Chi1) and glutamate 1-semialdehyde amino
transferase (GSA), both involved in chlorophyll biosynthesis, resulted in the
rapid and specific mRNA degradation upon induction with ethanol. Ethanol-
inducible silencing of the target genes caused strong but transient pheno-
typical alterations featured by a progressive loss of chlorophyll in young
leaves which persisted for about 7–9 days before newly growing leaves
completely recovered. Inducible gene silencing using the alc system promises
to ovivate the problems associated with constitutive RNA silencing and enables
the dissect primary and secondary effects of PTGS at temporal and spatial res-
olution.

FC195  The effect of tea catechins and theaflavins on UVB-induced cyclooxy-
ge nase-2 protein expression in HaCaT keratinocytes
University of Dundee, Dundee, United Kingdom.
Epidemiology has demonstrated that tea may have chemopreventive prop-
erties. Animal models have shown that tea and tea components such as
polyphenols can attenuate tumour development. Topically applied green tea
catechins can attenuate the effects of UVB in human and mouse skin.
Exposure of skin cells to UVB increases the transcription of many genes,
amongst them cyclooxygenase 2 (COX-2), and increased expression of this
enzyme has been associated with skin tumours. We have examined the effects of
a number of tea components from green tea (epicatechin gallate, epigallocatechin,
gallate, epigallochatechin gallate) and black tea (mixed theaflavins) on
UVB-induced upregulation of COX-2 expression in HaCaT keratinocytes.
Our results show that epicatechin gallate, and to a lesser extent epigallocate-
chin gallate attenuated UVB-induction of COX-2 protein expression. In con-
trast, black tea theaflavins increased COX-2 protein expression in the absence
of UVB, epigallocatechin gallate had a similar, but much less pronounced,
effect. Other phenols, such as alpha-tocoopherol, had no effect. The effects
of tea components on COX-2 protein expression did not correlate with their
effects on UVB-induced DNA damage or cell viability. Our results demonstrate
the different abilities of distinct tea compounds to modulate UVB effects in a
non melanoma skin cancer cell culture model.

FC196  New development in photoprotection: Oral supplementation in healthy vol-
umteers prevents the oxidative, inflammatory and immune UV-responses in
blood cells
C. Baudouin, M. Haure, C. Veissiere, M. Aries, M. Charverron;
Institut de Recherche Pierre Fabre, Toulouse, France.
Ultraviolet radiation is involved in skin damages including sun burn, pho-
toaging and photocarcinogenesis. The purpose of this study was to search
new ways for photoprotection and to evaluate an oral photoprotective prepa-
ration (PP), composed of antioxidants and essential fatty acids. In order to
mimic oxidative stress, we treated the peripheral blood lymphocytes of vol-
umteers by TNFalpha (50 ng/ml) or UVA (15 J/cm2) in vitro. Then, we analyzed
the activation of transcription factor NFkB and the generation of 8-oxo-7,8-
diethylene-2’-deoxyguanosine (8-OxodG) by HPLC/EC. Our results clearly show
the antioxidant properties of the oral PP supplementation. It significantly
decreased the UVA-induced 8-OxodG level and limited also the TNFalpha-
induced NFkB activation. As regards skin immunosuppression, we investigat-
ed the PP effect on monocyte-derived Langerhans cells (MoCs) generated in
vitro by precultivated monocytes of volunteers: we focused on the expression
of CD68 co-stimulatory molecule. FACScalibur flow cytometer analysis demo-
strated the restoration by the PP of the UVA-induced inhibition of CD68
expression illustrating its immunoprotective properties. This systemic photo-
protection limits the deleterious effects of UV for endpoints biological
responses that differ from sun burn. In combination with UV filters, this oral
supplementation could provide better photoprotection for human skin rele-
vant to prevent photocarcinogenesis and photoaging.

IL199  Reprogramming leaf metabolism by pathogens
U. Sonnewald, H. Tschiersch, E. Glickmann, F. Börnke, S. Biemelt;
Institute for Plant Genetics and Crop Plant Research, Gatersleben, Germany.
For successful parasitism of plants by fungal pathogens, it is apparent that
pathogens have evolved strategies for manipulating source-to-sink relations
and photosynthetic characteristics of the host plant. This complex interaction
involves fungal effectors which interact with their respective host factors. Amongst others, these effectors lead to tran-
scriptional regulation of target genes. We are studying the interaction
tial effectors into plant cells. Amongst others, these effectors lead to tran-
scriptional regulation of target genes. We are studying the interaction
of these effectors into plant cells. Amongst others, these effectors lead to tran-
scriptional regulation of target genes. We are studying the interaction

IL200  
Tetrapyrrole induced photosensitization in photosynthetic organisms  
B.C. Grimm  
Institute of Biology/Plant Physiology, Berlin, Germany.

Tetrapyrrole induced photosensitization in photosynthetic organisms Inna Lermontova, Tania Mischina, Bernhard Grimm Institute of Biology/Plant Physiology, Humboldt University Berlin, Germany. Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany. Tetrpyrrole biosynthesis provides metabolites that serve as prosthetic groups of various proteins involved in transfer of energy and electrons or in sensing redox states. The pathway is tightly regulated at several levels to coordinate apoprotein synthesis with cofactor availability and to avoid the accumulation of photosensitising intermediates. The metabolic control can be perturbed in photodynamically damaged mutant and transgenic plants with deregulated tetrpyrrole biosynthesis, by feeding of precursor or by the application of peroxidising herbicides, which inhibit protoporphyrinogen oxidase. We currently analyse mutant and transgenic plants with genetic lesions in various steps of (Mg)porphyrin biosynthesis. These plants suffer from accumulation of unbound photoreactive porphyrins in plants. In light, these unbound tetrapyrroles result in massive formation of reactive oxygen species that trigger a cascade of deleterious reactions in the photosensitised plants. Biochemical and genetic studies of these porphyric plants grown under various environmental conditions were performed to elucidate regulatory and protective mechanisms at the level of transcriptional activation, detoxification reactions of reactive oxygen species and porphyrin degradation processes.

IL201  
Reverse Genetics Of The Plant Light Harvesting Antenna  
S. Jansson1, J. Andersson1, U. Ganeteg1, C. Kühllein1, E. Boekema1, J. Dekker1  
1Umeå University, Umeå, Sweden, 2University of Groningen, Groningen, Netherlands, 3Vrije Universiteit, Amsterdam, Netherlands, 4University of Sheffield, Sheffield, United Kingdom, 5Uppsala University, Uppsala, Sweden.

We have analysed a set of Arabidopsis plants lacking the proteins Lhca1, Lhca2, Lhca3, Lhca4, Lhca5, Lhcb1/2 (LHC II), Lhcb4 (CP29), Lhcb5 (CP26), Lhcb6 (CP24) and PsbE as well as a set of Arabidopsis plants harboring an overexpression of the LHC II transporter T-DNA tagging. Removal of the target protein led sometimes to a decreased stability of other proteins, most likely proteins in direct contact with each other. Electron microscopy gave structural information and showed that in plants lacking Lhcb1 and Lhcb2, proteins that make up the LHC II trimers, Lhcb3- and CP26-containing trimers replaced the LHC II trimers in the PS II supercomplex, preserving PS II ultrastructure in the absence of LHC II. These plants were capable of forming stacked membrane, but not to perform state transitions and grew poorly under low light. The other lines were also analysed in terms of photosynthetic function. To assess the importance of each individual protein, we have grown the Arabidopsis lines in the field and analysed their seed production, which in case of Arabidopsis should equal fitness. The interpretation of the results is that each protein has a unique role in the photosynthetic apparatus that not could be taken over by the other proteins.

IL202  
Redox regulation of thylakoid protein phosphorylation  
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University of Turku, 2004 Turku, Finland.

The photosystem II of chloroplast thylakoid membranes contains several proteins phosphorylated by redox-activated protein kinases. The mechanism of the reversible activation of the light harvesting antenna complex II (LHCCI) kinase(s) is one of the best understood and related in short term to the regulation of excitation energy distribution between photosystems II or I (State Transition). The deactivating LHCCI protein kinase(s) is associated with cytochrome b/f and dissociates from the complex upon activation. Activation of the LHCCI protein kinase occurs via dynamic conformational changes in the cytochrome b/f complex taking place during plastoxinol oxidation. Deactivation of the kinase involves its reassociation with an oxidized cytochrome complex. A fine-tuning redox-dependent regulatory loop inhibits the activation of the kinase at high irradiances via reduction of protein disulphide groups catalysed by respective thioredoxins. The reversible phosphorylation of LHCCI and other thylakoid phosphoproteins, catalyzed by respective kinases and phosphatases, is under strict regulation in response to environmental changes. We have also studied whether the activation, deactivation or inhibition of thylakoid kinase(s) is involved in redox-mediated crosstalk between the chloroplasts and the nucleus by monitoring nuclear gene expression with Arabidopsis cDNA microarrays under conditions which induce LHCCI kinase activation, deactivation or inhibition.

IL203  
Role of chloroplast derived ROS in plant defense  
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University of Helsinki, Helsinki, Finland.

Accumulation of ROS (reactive oxygen species) is central to plant response to pathogens and UV-B. However, the role of ROS in plant defense is complex and not well understood. Accumulation of ROS in plants is a result of a number of pathogens, such as tobacco mosaic virus, and UV-B radiation. The role of ROS in defense related to pathogen attack or UV-B is not clear. There are several sources of ROS involved in the plant responses and PCD including the plasma membrane (NADPH oxidase complex), mitochondria and chloroplasts. We have elucidated the role of chloroplasts in defense-related ROS production and its consequences to plant resistance. Chlorophyllase (encoded by AtCLH1 in Arabidopsis) is central to chlorophyll degradation and removal of chlorophyll after tissue damage e.g. due to pathogen attack. Expression of AtCLH1 was strongly induced in response to the bacterial necrotroph Erwinia carotovora. To elucidate the role of chlorophyllase in plant defense the gene was silenced by RNAi in Arabidopsis. Silencing of the AtCLH1 gene led to light-dependent resistance to E. carotovora. These plants accumulated hydrogen peroxide in response to E. carotovora and exhibited increased expression of genes related to systemic acquired resistance (SAR). In contrast, the RNAi silenced plants showed decreased resistance to the necrotrophic fungus Alternaria brassicicola, where plant resistance requires induction of jasmonate (JA) dependent defenses. We propose that inactivation of chlorophyllase enhances ROS production and SAR response but downregulates JA-dependent defense responses.

IL204  
ROS turnover and oxidative damage to proteins in plant mitochondria  
I. Møller  
Risø National Laboratory, Roskilde, Denmark.

The first part of the lecture will be a review of the three-step strategy used to deal with reactive oxygen species (ROS) – Avoidance, Detoxification and Repair (IM Møller 2001. Annu.Rev. Plant Physiol Plant Mol Biol 52, 561-591). ROS are produced by the respiratory chain in the presence of oxygen probably mainly in complexes I and III and mainly when the electron transport components are more reduced. ROS production can therefore be limited by mechanisms preventing over-reduction, e.g., the alternative oxidase. If ROS production cannot be avoided, the ROS can be detoxified by several different mechanisms. When the first two strategies – avoidance and detoxification – are not sufficient, ROS accumulate. As a result, damage occurs to lipids (mainly to polyunsaturated fatty acids), proteins and DNA and this damage must be repaired. We are studying the oxidative modification of proteins by tagging oxidized proteins with dimethylphenylhydrazine (DMPH). The tagged proteins can then be localized on 2D Western blots or immunoprecipitated using a DNP-specific antibody and the proteins identified by trypsin digestion followed by mass spectrometry. The results of a model study and a physiological study will be presented.
IL206
Reactive oxygen species generated by UVA in mammalian cells – the role of iron
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University of Bath, Bath, United Kingdom.

The ultraviolet A (320–380 nm) component of sunlight generates an oxidative stress in skin which contributes to both the acute (sunburn) and chronic (aging, skin cancer) effects of sunlight. The damaging effects occur via generation of active oxygen species and will be exacerbated by the presence of catechol-actively reactive iron so that the observation that UVA radiation causes an immediate release of “free” iron in human skin fibroblasts and keratinocytes via the proteolysis of ferritin is likely to be biologically significant. UVA radiation also breaks down heme-containing proteins in the microsomal membrane to release heme. The well-characterised activation of heme oxygenase 1 by UVA radiation will lead to breakdown of heme and further release of free iron. Overall these interactions generate a strong oxidative stress on cells. Both the basal and UVA-induced levels of labile iron are 2–4 times higher in fibroblasts than keratinocytes and this is consistent with the higher resistance of keratinocytes to UVA-induced necrotic cell death. Modulating cell iron levels by hemin (to enhance the levels) or iron chelators (to reduce the levels) has the predicted effect on levels of necrotic cell death. Overall these studies further illustrate the potent oxidising nature of UVA radiation.

IL207
Cytoprotective and cytotoxic effects of in vivo flavonoid metabolites in human skin fibroblasts under oxidative stress
C. Rice-Evans;
Wolfson Centre for Age-Related Diseases, London, United Kingdom.

In delineating the cytoprotective effects of flavonoids against oxidative stress in human skin fibroblasts, few studies have considered the influence of gastrointestinal and hepatic metabolism and the consequences of the ensuing chemical structural changes on their biological properties and activities. Thus the ability of flavonoids to protect cells against oxidative stress-induced cell death might occur via mechanisms independent of conventional hydrogen-donating properties, namely, involving cell signalling and changes in gene expression. These proposals are based on the observations that, firstly, flavonoids are metabolised on absorption thus influencing the structural features governing their antioxidant properties, and, secondly, that circulating levels of flavonoids are observed in the low micromolar range. Hence, these pre-set the factors influencing the differential effects of three major classes of flavonoids, the flavonols – represented by quercetin, the flavonans – represented by epicatechin, and the flavanones – represented by hesperetin, and their corresponding in vivo conjugates and metabolites, in protecting human skin FcK4 fibroblasts from cell death induced by oxidative stress. Modifications to flavonoids from aglycone to in vivo forms increasing polarity and lack of intracellular access through glucuronidation, on the one hand, and decreasing polarity and as well as modulation of redox properties through methylation of catechol moieties, on the other hand. The results show that epicatechin protects human skin fibroblasts against apoptosis induced by oxidative stress through mechanisms involving the modulation of JNK-activation. Interestingly, its methylated forms (with substituted catechol structures) are equally efficacious suggesting the lack of requirement for the redox-active catechol moiety in its mechanism of action. The glucuronide, however, is ineffective, suggesting a requirement for intracellular access or that the A-ring, modified on glucuronidation, might mediate specific binding.

IL208
Oxidative damage and cytotoxicity of UVA depends on the mode of exposure
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Vienna Medical School, Vienna, Austria.

The influence of UV dose fractionation on photochemical effects is largely unknown. In our experiments we compared the effects of fractionated vs single dose UVA at a constant total dose on viability, lipid peroxidation and the generation of reactive oxygen species in the human squamous carcinoma cell line A 431. Lipid peroxidation was measured by TBARS formation and ROS levels. In our experiments we compared the effects of fractionated vs single exposure with intervals of 10, 30 and 60 min. Fractionated exposure with intervals of 10, 30 and 60 min led to an increase in viability of up to 40% compared to the identical single dose. With longer intervals, in contrast, the survival rate increased of up to 30%. Measurement of TBARS and DCFH-DA fluorometry showed that with short intervals oxidative damage increases from fraction to fraction. The amount of ROS and TBARS generated by the third fraction of 20J/cm² exceeds that generated by 60J/cm² in cells without prior exposure. These results indicate that repeated exposure with short intervals exhaust the antioxidative capacity of cells and leads to accumulation of oxidative damage and increased cell death.

IL209
Detection of reactive oxygen species based on chemiluminescence of luminal and Cypridina Luciferin Analogues
M. Domínguez-Cherit, J. Snýdcchová;
Palacky University, Olomouc, Czech Republic.

In recent years, considerable interest has been focused on the reactions of reactive oxygen species with organic compounds and their toxicity towards living cells. Known as O₂- generating systems such as NaOCl-H₂O₂, myeloperoxidase-H₂O₂-halide ions and light-photosensitizer-O₂ systems contain reactive oxygen species other than O₂- and often also generate free radicals. In connection with the mechanism of bioluminescence of Cypridina luciferin/ Cypridina luciferase system, the chemiluminescence of various Cypridina luciferin analogues is intensively investigated. 2-Methyl-6-phenyl-3,7-dihydroimidazol/1,2-a/pyrazin-3-one (CLA) or 2-methyl-6-(methoxyphenyl)-3,7-dihydroimidazol/1,2-a/pyrazin-3-one (MCLA) react with O₂- or 1O₂ to emit light. The luminal-H₂O₂-CuSO₄, CLAs-H₂O₂-CuSO₄, luminal-H₂O₂-HRP, CLAs-H₂O₂-HRP, luminal-NOCl-H₂O₂, and CLAs-NOCl-H₂O₂ systems were studied at 25°C and 37°C. Superoxide dismutase (SOD, a scavenger of O₂-) and NaN₃ (a quencher of 1O₂) were used for differentiation between O₂- and 1O₂-dependent luminescence.

IL210
Kinetic behavior of singlet oxygen in simple models of biochemical systems.
Laser-induced oxygen phosphorescence in water; dependence on detergents and sodium azide
A. A. Krasnoyarsky, D. Buturina;
N. B. Institute of Biochemistry, Moscow, Russian Federation.

The kinetics of photosensitized phosphorescence of singlet molecular oxygen (SO) after pulsed laser excitation and dependence of these parameters on sodium azide were studied in air-saturated porphyrin solutions in ethanol and water. Without azide, the rise of the phosphorescence intensity after the laser pulse reflected the time course of energy transfer from triplet porphyrin to oxygen, the decay – SO deactivation. The rise times were 0.4±0.1 µs in ethanol and 2.0±0.2 µs in water. The lifetimes of SO decay were 13±1 µs in ethanol and 3.15±0.2 µs in water. If 1–20% detergents were added to water, the SO decay time increased to 4.5–8 µs. The data suggest that, in detergent solutions, SO molecules are mostly located in lipophilic micelles and not in water. Sodium azide decreased the phosphorescence intensity. The rate constants of SO quenching strongly depended on solvents and detergents and varied from 2 to 12·10⁶ M⁻¹·s⁻¹. With the high azide concentrations, when the SO lifetimes were near those in the structures of living cells, the phosphorescence kinetic phases occurred. The rise corresponded to SO deactivation and the decay, to the transfer energy from triplet porphyrin to oxygen. Similar kinetic behavior is expected also in biological systems.

IL211
Sunlight forms cyclobutanopyrimidine dimer, but not (6-4) photoproduct nor 8-hydroxydeoxyguanosine in broad bean leaves
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In contrast to DNA lesion caused by artificial UVB almost no data is available on the effects of sunlight on DNA lesion in plants. To clarify the obscurity we exposed leaf disks of broad bean (Vicia faba L.) to light in two culture sections, respectively, receiving full-band-sunlight (+UVB) and UVB-cut-off-sunlight (–UVB), and followed the variation in amount of DNA lesion products. The experiment started at noon and ended at 14:30 h on the next day. In +UVB the level of cyclobutanopyrimidine dimer (CPD) increased till near the sunset at 18:00 H, then decreased slightly in the dark till 09:00 H in the next morning, and then increased further during 5.5 h in +UVB to surpass the highest value on the first day. In –UVB no CPD was formed. When leaf disks kept in +UVB on the first day was transferred to –UVB, the CPD formed on the first day disappeared completely during 5.5 h of the second day. These results indicate that UVB in sunlight forms and accumulates CPD, and the waveband other than UVB is effective in repairing the accumulated CPD. No formation of (6-4) photoproduct and 8-hydroxydeoxyguanosine was noted in this experiment.
Formation of inter-strand bipyrimidine photoproducts within UVC-irradiated DNA

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Dimerization of adjacent pyrimidines has been identified as the main genotoxic process triggered by far-UV radiation. The resulting lesions include cyclobutane dimers and (6-4) photoproducts, together with 5,6-dihydro-5-(alpha-thyminyl)-thymine, the "spore photoproduct", which is specifically formed in dehydrated DNA. We report a comparison of the distribution of bipyrimidine photoproducts within DNA exposed to UVC in aqueous solution with that determined within DNA specifically set in the A-form either by addition of ethanol or in the dry state. The quantification of the bipyrimidine lesions was based on the HPLC-tandem mass spectrometry analysis of enzymatically digested samples. In addition to the formation of the spore photoproduct, a specific feature of the photochemistry of A-DNA was found to be the induction of photoproducts involving thymine bases located on opposite strand of the duplex. Indeed, the dinucleoside derivatives of various diastereoisomers of the spore photoproduct, cyclobutane dimer and (6-4) adduct were detected in large amount following enzymatic digestion. The latter compounds did not arise from lesions involving adjacent pyrimidines that are released as modified dinucleoside monophosphates. Interestingly, A-DNA is predominant in bacterial spores. Therefore, the formation and repair of the inter-strand photoproducts in the latter micro-organisms would be a topic for further investigations.

Role of UV(A) induced DNA double-strand breaks (DNA-dsb) as precursor lesions of chromosomal aberrations important for skin cancer development

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Chromosome aberrations are known to be important etiological factors in cancer development. This holds also for skin cancer, where chromosomal aberrations have been shown to appear in certain stages of tumour development. This is, to our knowledge, the first experiment which shows that UV-irradiation, at physiologically relevant doses, is able to induce DNA-dsb (precursor lesions for chromosome aberrations) in entire cells. Our results will be discussed in the context of mechanisms of UV-induced skin cancer and the use of chromosomal markers for risk assessment.

Non-lethal DNA damage in non-melanoma skin cancer cells induced by Photofrin-PDT: A comet assay study

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Photodynamic therapy (PDT) uses a photosensitising chemical, oxygen and visible light to kill tumour cells. Photo-oxidation of the cell membrane plays a major role in the mechanism by which cell death occurs. DNA is also damaged, but this is considered to be a late event and does not contribute to the phototoxic effect. The relevance of PDT-induced DNA damage to the clinical situation is unclear, but as PDT is now being proposed not only for the treatment of cancer, but also for benign conditions, it is important to establish whether DNA damage will have significant consequences. To achieve our primary objective, we used the single cell gel electrophoresis assay (Comet assay) in combination with DNA repair enzymes to show that Photofrin-PDT increases frank DNA strand breaks and oxidative base damage at low doses in HaCaT keratinocytes. Strand breaks were detected at an early stage, prior to loss of cell viability and could be attenuated by antioxidants. However, antioxidants had no effect on acute phototoxicity elicited by Photofrin-PDT. We also demonstrated that treatment with Photofrin in the dark increased DNA base oxidation without increasing DNA strand breakage.
Immunological effects of acute and chronic UVA exposures

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It is well established that UVB (290–320 nm) has immunosuppressive effects that may be involved in skin cancer in humans. Relatively few studies have examined the effects of physical doses of UVA (320–400 nm) on in vivo human skin immunity. Some investigators have used contact-hypersensitivity or delayed-type-hypersensitivity responses as models to study UVA-induced immunosuppression; however, as immunological end-points, most studies have investigated the alterations of cutaneous immunocompetent cells. As assessed by the contact hypersensitivity model, UVA has been shown to suppress both primary and secondary immune responses. It has been demonstrated that UVA exposure suppress also delayed type hypersensitivity responses to recall antigens. At cellular level, UVA exposure alters the epidermal-dermal network and effectively depletes Langerhans cells and epidermal T cells from the irradiated skin. In contrast, UVA radiation is a poor inducer of suppressive macrophages. Taking together, as UVA also UVB wavebands are immunosuppressive and the latter is relatively more immunosuppressive than UVA, UVA induce a transient immune-suppression, whereas UVB has more sustained effects. Finally, exposure to UVA may have other implications for human health since the immune protective effects of UVA (340–400 nm) observed in murine studies have been suggested also in humans.

Bacteriorhodopsin (BR) is a photochromic protein found in Halobacterium salinarum. Its biological function is that of a light-driven proton pump which converts energy from sunlight into chemical energy. Due to its attractive physical functions and the easy availability of the material several technical applications have been proposed, many of them in the optical field. Genetic engineering of the halobacteria allows to design and to produce modified BRs with optical properties optimized for the different applications. A short review on the area of optical applications of BR will be presented and some newer developments will be described in more detail, e.g., holographic interferometry with optical films made from BR and photosensitive BR-based pigments for anti-counterfeiting applications.

**Abstracts**

**II.218 Cellular-molecular effects of UVA**

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Today there is an ever-increasing appreciation of the importance of UVA effects on cellular and molecular responses. For a long time UVA was seen as an innocent bystander in many biological effects of sunlight. However, the important role of UVA in many cutaneous photosensitivity diseases has strongly indicated its potential as hazardous agent. At present it is possible for people to expose themselves to very high doses of UVA in a short time, doses that were not commonly reached in the past. Some cellular molecular effects of UVA, such as DNA damage, matrix metalloproteinase activation, induction of apoptosis, haem oxygenase expression are well defined but the chromophores for most reactions are unknown. In contrast to direct and indirect induction of apoptosis, haem oxygenase expression are well defined but the effects of UV, such as DNA damage, matrix metalloproteinase activation, are in use today are UVA-emitting (315–400 nm) appliances that are classified or UVB-dominated sources poses less risk.

PICTODININE didecylide: a new DNA photo protector for human skin

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Thymidine didecylide (pT) stimulates photo protective responses in mammalian cells and intact skin, through increasing melanogenesis and enhancing DNA repair induced by UV light. UV is associated with decrease in DNA repair systems which is related with decreased constitutive levels of nucleotide excision repair proteins. We have used UV treated skin from donors were pretreated with thymidine didecylide (pT) for 24 h, then UV-irradiated with solar-simulated light. Evaluation was done by various techniques such as; DNA fragmentation by gel electrophoresis, protein expression and I NOS by PCR and Scanning electron microscopy and they revealed that pretreatment with pT up regulated protein expression at 24, 48, and 72 h. Also, pT enhanced repair of DNA damage done by UV light. Our studies suggest that topical pT treatment may enhance repair capacity in adult skin and thus may be used as a solar UV photo-protective.

**II.222 Applications of Bacteriorhodopsin in Optical Information Processing**

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Bacteriorhodopsin (BR) is a photoreactive protein found in Halobacterium salinarum. Its biological function is that of a light-driven proton pump which converts energy from sunlight into chemical energy. Due to its attractive physical functions and the easy availability of the material several technical applications have been proposed, many of them in the optical field. Genetic engineering of the halobacteria allows to design and to produce modified BRs with optical properties optimized for the different applications. A short review on the area of optical applications of BR will be presented and some newer developments will be described in more detail, e.g., holographic interferometry with optical films made from BR and photosensitive BR-based pigments for anti-counterfeiting applications.

**II.226 Applications of Bacteriorhodopsin in Optical Information Processing**

A. Maass
Applications of Bacteriorhodopsin in Optical Information Processing

**II.227 Optical Data Storage using peptides**

R. H. Berg, P. S. Ramamujam, Riso National Laboratory, Roskilde, Denmark.

Optical storage represents unique opportunities for storing data at high density. It is believed that organic materials will be used extensively in future data storage systems. However, the fact is that optical storage technology presently suffers from an absence of practical organic materials that apply to the next generation of commercially available laser diodes, namely the blue ones that operate between 400 and 500 nm. Looking further into the future, ultra-high density optical storage will move towards the use of even shorter wavelengths. Here, we present peptides that may be useful as future materials for optical data storage. As an example, a large refractive index change can be obtained in blue and UV region through photodimerization of neighboring chromophores. The photodimerizable chromophores are attached to a short piece of peptide, such as DNO developed for optical storage at visible wavelengths. The Thymine containing peptides have been synthesized. Information optically stored in films of such peptides has proved to be extremely stable.
Control of energy and electron flow in artificial photosynthetic antennas and reaction centers

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Artificial antenna systems and reaction centers synthesized in our laboratory are the building blocks of nascent artificial photosynthetic assemblies that could be used for devising cell-like constructs in which complex biological reactions and processes are supplied with energy and controlled. In cells, the control of energy and electron flow in response to ambient conditions is essential to life and will be necessary in artificial systems that dissipate energy and carry out work. In nature, excitation energy flow in photosynthetic membranes is controlled by several mechanisms including quenching of tetrapyrrole excited states by carotenoid pigments. From model studies of carotenoids linked to tetrapyrroles we have found essential structural features that control electronic coupling and thermodynamics and thereby control the energy and electron transfer processes that can quench tetrapyrrole excited states. Artificial reaction centers have been designed and synthesized in which photoinduced electron transfer giving rise to primary charge separation is controlled by switching energy transfer on or off according to the state of an attached photochromic moiety. This work has been further elaborated to include the control of charge separation by the redox state of an attached switching element. Progress towards incorporating artificial antennas and reaction centers fitted with these control elements into model biological membranes where they should be able to control ion pumps and therefore membrane potentials and bioenergetic processes will be reported.

Environmental pathology – impacts of ozone depletion and climate change

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Photobiological processes in the environment are many and varied. They can affect human health, aquatic and terrestrial ecosystems, air quality, and man-made materials. The processes are typically driven by solar UV-B radiation and are therefore subject to changes caused by changes in atmospheric ozone. Until recently the issue of ozone depletion was considered separately from other issues of climate change, such as global warming due to increasing greenhouse gases. This approach is now considered over simplistic and in recent years there has been a growing awareness of the importance of interactions between ozone depletion and global warming. These interactions can involve chemistry, radiation, and transport. The interactions are sometimes quite complex and the feedbacks can be positive or negative. Here we discuss how these interactions can exacerbate or ameliorate the photobiological processes. The main focus is on the future impacts on ozone of increasing greenhouse gases (GHGs), and how these impacts may change as a result of measures to control GHG emissions, and what we can do to monitor, understand, and mitigate these effects.

Expression and regulation of cytochrome P450 CYP2S1 in human skin by ultraviolet radiation and therapeutic agents for psoriasis

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Inter-individual differences in response to topical agents are a significant clinical problem. Individuality in hepatic drug metabolising enzyme expression is an important determinant of systemic drug handling; variation in cutaneous gene expression may therefore contribute to individuality in response to topical therapies. A novel P450, CYP2S1, has recently been described in extra-hepatic tissues (Rylander et al. Biochem Biophys Res Commun 2001; 281: 529–535) but has not been examined in skin. We investigated CYP2S1 regulation by ultraviolet radiation in skin and patients with psoriasis. We also investigated CYP2S1 regulation by ultraviolet radiation in skin and patients with psoriasis. We also investigated CYP2S1 regulation by skin. We demonstrated that CYP2S1 is expressed in human skin, and how these impacts may change as a result of measures to control GHG emissions, and what we can do to monitor, understand, and mitigate these effects.
The Digital Photobiology Compendium: Overview and evaluation

The treatment of hand dermatitis is difficult. Limited short-term improve-
ments have been described following UV phototherapy. In addition, UV radia-
tion is a complete carcinogen which renders this treatment inappropriate for
the longterm management of children and young adults. As a new theraeup-
tic modality, DermoDyne® UV-free phototherapy has been tested for treat-
ment in 15 patients with chronic hand or foot dermatitis. The patients were
first treated with a sham irradiation device. Thereafter, they received 12
DermoDyne® treatment for another four weeks. Over 40% of the radiation is
being emitted between 400-500 nm. The residual UVA-2 emission was less
than 1 W/cm² per treatment, and thus, by definition (ICNIRP/IRPA).

DermoDyne® therapy is UV-free. The Font face=Arial>sham irradiation did not
cause any improvement. One patient dropped out because of worsening
of eczema. The DASI score was reduced by 37% (p<0.01), 52% (p<0.001), and
86% (p<0.001) after the first, second and fourth week respectively.

Patient evaluation three months after the end of the treatment still showed
a mean DASI score reduction of 81% (p<0.001). This study demonstrates
that DermoDyne® appears to be an effective UV-free phototherapeutic treat-
ment for patients with chronic palmoplantar eczema. In contrast to other
treatments DermoDyne® appears to have long-term efficacy.

FC238
Zinc octa-decyl phthalocyanine: a candidate for photodynamic treatment of
psoriasis

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1Institut fuer Umweltmedizinische Forschung (IUF) at the Heinrich-Heine-University (H-H-U), Duesseldorf, Germany, 2Dept.
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The dye 1,4,8,11,15,18,22,25-octakis(decyl)phthalocyaninato zinc(II) (ZnOOPC)
was preclinically investigated for its use in photodynamic therapy, namely
as a potential drug for the treatment of psoriasis. ZnOOPC exhibited
favourable properties as a photosensitiser in vitro: absorption maxima of 700
nm with an extinction coefficient of 191 000 (M-1 cm)-1, a singlet oxygen
quantum yield of 0.47 ± 0.02 , and a good accumulation in a variety of cuta-
necous and subcutaneous fibroblast cultures. In vivo experiments were
performed on the skin of healthy mice. These experiments demonstrated a
good accumulation of ZnOOPC after topical application to the skin of mice.
From these results, ZnOOPC was considered as a candidate for clinical
investigations.

In another series of experiments we investigated the therapeutic
potential of ZnOOPC for the treatment of psoriasis. A high dose of
ZnOOPC (100 mg/kg) was topically applied to the skin of mice. This dose
resulted in less than 1 J/cm² per treatment, and thus, by definition (ICNIRP/IRPA).

In the present study we investigated the therapeutic potential of
ZnOOPC for the treatment of psoriasis in vivo. This approach is based
on the photothermal properties of ZnOOPC. The treatment was repeated
every second day for 4 consecutive weeks. After each treatment the
mice were evaluated for clinical signs of inflammation.

The results demonstrated that ZnOOPC is a promising candidate for
photodynamic treatment of psoriasis. Further studies are necessary to
determine the optimal dose and treatment regimen to achieve maximal
therapeutic effects.

The study was supported by the Photobiology Educational and Research
Foundation and the US Department of Education (FIPSE). Contents do not
necessarily represent the policy of the Department of Education, and you
should not assume endorsement by the Federal Government.
The presence of plasma. This can be overcome when photodynamic treatment with thionine (Th). MB is used to decontaminate plasma and Th for treatment of other viruses, e.g. parvovirus B19. In addition, it may contain bacteria. This is especially critical for platelet concentrates (PC) because they have to be stored at 20–24°C, temperatures at which bacteria can multiply to high levels. In the meantime the bacterial risk of blood components is regarded as negative bacterium, which may be influenced by the presence of ions in the plasma. This can be overcome when photodynamic treatment is followed by short irradiation with UVB-light (Fl 1 Joule/cm²). Other phenothiazine dyes exhibit higher antimicrobial activity than MB or Th, but platelets and their functions are also negatively affected.

The safety of therapeutic blood products is based mainly on the advanced test systems used to detect markers of viruses, i.e. HIV and the hepatitis viruses B and C, respectively. Donated blood may, however, be contaminated with other viruses, e.g. parvovirus B19. In addition, it may contain bacteria. It is well established that for successful photoinactivation (PI) of gram-negative bacteria a cationic photosensitizer is required. This requirement suggests a charge-dependent interaction between the photosensitizer and the gram-negative bacterium, which may be influenced by the presence of ions in the suspending medium. The aim of the present study was to investigate the effect of cations, Na⁺ and Ca²⁺, in the suspending buffer on the efficacy of the PI of the gram-negative Pseudomonas aeruginosa and the gram-positive Staphylococcus aureus. The bacteria were sensitized in HEPES buffer containing either meso-tetra(N-methyl-4-pyridyl)-porphyrin or meso-monophenyl-tri(N-methyl-4-pyridyl)porphyrin as photosensitizer and various concentrations of Na⁺ or Ca²⁺. The cell suspensions were then exposed to a broadband light dose of 9.1 cm-2 and the number of surviving bacteria was determined. In HEPES buffer without added cations, P. aeruginosa and S. aureus were equally sensitive towards PI. Addition of cations strongly decreased the sensitivity of both bacteria towards PI, with the PI of P. aeruginosa being much more decreased than that of S. aureus and Ca²⁺ being more effective than Na⁺.

### IL245

**Photodecontamination in blood products by thionine and other phenothiazine dyes**

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Blood Center of the German Red Cross, Springer, Germany.

The safety of therapeutic blood products is based mainly on the advanced test systems used to detect markers of viruses, i.e. HIV and the hepatitis viruses B and C, respectively. Donated blood may, however, be contaminated with other viruses, e.g. parvovirus B19. In addition, it may contain bacteria. It is well established that for successful photoinactivation (PI) of gram-negative bacteria a cationic photosensitizer is required. This requirement suggests a charge-dependent interaction between the photosensitizer and the gram-negative bacterium, which may be influenced by the presence of ions in the suspending medium. The aim of the present study was to investigate the effect of cations, Na⁺ and Ca²⁺, in the suspending buffer on the efficacy of the PI of the gram-negative Pseudomonas aeruginosa and the gram-positive Staphylococcus aureus. The bacteria were sensitized in HEPES buffer containing either meso-tetra(N-methyl-4-pyridyl)-porphyrin or meso-monophenyl-tri(N-methyl-4-pyridyl)porphyrin as photosensitizer and various concentrations of Na⁺ or Ca²⁺. The cell suspensions were then exposed to a broadband light dose of 9.1 cm-2 and the number of surviving bacteria was determined. In HEPES buffer without added cations, P. aeruginosa and S. aureus were equally sensitive towards PI. Addition of cations strongly decreased the sensitivity of both bacteria towards PI, with the PI of P. aeruginosa being much more decreased than that of S. aureus and Ca²⁺ being more effective than Na⁺.

### IL244

**Antimicrobial Photodynamic Therapy: state-of-the-art**

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Photodynamic Therapy (PDT) appears to be endowed with several favourable features for acting as an alternative to antibiotic treatment of microbial infections, including those chronicized after chemotherapy. This field is increasingly challenged by the rapid evolutionary changes and large number of multidrug-resistant pathogens. The mechanism of action of many photodynamic sensitisers on microbial cells is substantially different from that typical of antibiotics, while the photosensitivity of microorganisms is generally independent of their antibiotic resistant spectrum. Moreover, an appropriate control of the parameters involved in the PDT protocol allows one to achieve an extensive decrease in the pathogen population at the target site with minimal damage on the hosted tissue and no adverse consequences for the normal “friendly” flora. Optimal results are obtained by using cationic photosensitisers belonging to the class of phenothiazines, porphyrins and phthalocyanines, which cause an efficient inactivation of Gram-positive and Gram-negative bacteria, yeasts, micoplasmas and parasites (at the stage of both of cysts and vegetative cells). Present indications suggest that PDT can become a mainstream option for the treatment of localized infections, such as oral candidiasis, periodontal diseases and healing of infected wounds.

### IL247

**Photodynamic therapy and skin mycosis**

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The yeast-like fungus candida albicans can normally be found on mucous membranes, gastrointestinal tract, vaginal vault and skin. Under certain circumstances, it can change from a commensal organism to a pathogen and cause localized or generalized mucocutaneous disease. Candidiasis is not uncommonly resistant to standard therapies and recurrences are frequent. Photodynamic anti-microbial chemotherapy (PACT) of candida infections has been recently investigated in “in vitro” studies. The results of experimental investigations have demonstrated that candida albicans can be sensitized at a variable extent by a number of dyes, i.e. toluidine blue, methylene blue, chlorins, phthalocyanine, diaminoo-acid derivatives of porphyrins and haematoxyphyrins. The subsequent irradiation with visible, either coherent or polychromatic, light causes variable degrees of cell death. However, a large number of experimental conditions, e.g. dye and cell concentration, cell size/volume, emission spectra, light energy density and irradiance, period of incubation before and after irradiation, can deeply affect the results of “in vitro” assay system. The mechanism of pact destruction of yeasts involves the photodamage of cytoplasmic enzymes, membrane lipids an c nucleic acids leading to cell death.

### FC247a

**Effect of Na⁺ and Ca²⁺ on the photoinactivation of bacteria with cationic porphyrins**

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It is well established that for successful photoinactivation (PI) of gram-negative bacteria a cationic photosensitizer is required. This requirement suggests a charge-dependent interaction between the photosensitizer and the gram-negative bacterium, which may be influenced by the presence of ions in the suspending medium. The aim of the present study was to investigate the effect of cations, Na⁺ and Ca²⁺, in the suspending buffer on the efficacy of the PI of the gram-negative Pseudomonas aeruginosa and the gram-positive Staphylococcus aureus. The bacteria were sensitized in HEPES buffer containing either meso-tetra(N-methyl-4-pyridyl)-porphyrin or meso-monophenyl-tri(N-methyl-4-pyridyl)porphyrin as photosensitizer and various concentrations of Na⁺ or Ca²⁺. The cell suspensions were then exposed to a broadband light dose of 9.1 cm⁻² and the number of surviving bacteria was determined. In HEPES buffer without added cations, P. aeruginosa and S. aureus were equally sensitive towards PI. Addition of cations strongly decreased the sensitivity of both bacteria towards PI, with the PI of P. aeruginosa being much more decreased than that of S. aureus and Ca²⁺ being more effective than Na⁺.

### FC246

**Phthalocyanines as useful PDT antimicrobial agents**

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**Phthalocyanines as useful PDT antimicrobial agents**

Romucci G.1, Chiti G.1, Deli D.3, Fantetti L.1, Jori G.2;

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As widely recognized, the large variety of pathogens as well as the resistance of bacteria to antibiotic treatment are main factors of concern in the realm of bacterial infections. The classic approach is to increase the dosage or to prescribe new types of antibiotics, however the gap between the problem and its solution needs to be closed yet. In particular, the spread of antibiotic resistance fueled by the extensive use of antibiotics has caused from one side the emergence of resistant strains and the urgent need for alternative therapeutic treatments from the other. Photodynamic Therapy (PDT), a bimodal strategy which relies on photosensitizers and light, may offer a new approach for the inactivation of microbial pathogens and phthalocyanines molecules appear to be photosensitizers of choice to develop into microbial agents. QSAR studies previously conducted for this class of molecules have established the important structural features for an efficient photosensitization of microbial pathogens. Variation of the chemical structure has permitted the identification of photosensitizers characterized by a broad as well as narrow spectrum of activity which is independent from the previously developed antibiotic resistance and show no genotoxicity. Some of these products are being tested in vivo in three animal models of infection with promising results.

### FC248

**Future prospects for photoantimicrobial therapy**

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The antimicrobial effects of photodynamic therapy have been known for over a century and yet it is the anticancer regimen which has received the greater attention. While this may be in part due to the availability and efficacy of standard antimicrobials, there has also been reluctance for researchers in the antitumor field to become involved with the antimicrobial application. However, the use of photodynamic disinfection protocols, such as those employed in blood products has demonstrated the ease of application of photosensitisers to microbial targets and is encouraging more general research into anti-infectives. This is particularly relevant in view of the increasing occurrence of drug-resistant bacterial infections in our hospitals, where the direct treatment of infected wounds and also the blocking of infection transmission is possible. The use of photosensitisers in the treatment of the microbially-diseased state (e.g. pulmonary tuberculosis) is also slowly gaining acceptance.
Photodynamic destruction of anthrax-like spores

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Anthrax spores (Bacillus anthracis) can be prepared in an aerosol and used in biological warfare and bioterrorism. Spore formation is a sophisticated mechanism by which some Gram (+) bacteria survive conditions of stress and starvation by producing a multilayered protective capsule enclosing their dehydrated and condensed DNA. Spores are highly resistant to damage by heat, radiation, and commonly employed anti-bacterial agents, and similar spores have been previously shown to be resistant to photodynamic inactivation (PDI) using dyes and light that easily destroy the corresponding vegetative bacteria. We have discovered that many species of Bacillus spores are inactivated by exposure to toluidine blue O (and to a lesser extent methylene blue) and red light. B. cereus spores (closely related to B. anthracis) were most sensitive (>99.9% were killed by 100 µM toluidine blue and 60 J/cm² of 635-nm light). B. subtilis spores were somewhat less sensitive while B. atrophaeus were least sensitive. PDI was still effective after spore suspensions were centrifuged and resuspended, implying the dye bound to and penetrated into the spore. Under certain circumstances B. cereus spores were more sensitive to PDI than vegetative cells. This relatively mild procedure to kill spores may be applicable when tissue has been contaminated with spores (e.g. in battlefield wounds).

The effect of iridial melanin on formation and decay of reactive oxygen species

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The amount of light that reaches the retina is mainly controlled by the pigmented iris. Iridial melanin, being exposed to significant fluxes of solar radiation, including its ultraviolet components, could contribute to generation of reactive oxygen species and undergo photochemical modifications. In this study, we examined the type and content of iridial melanin in human eyes of different iris color and donors age using electron paramagnetic resonance (EPR) and HPLC detection of characteristic products of melanin degradation, the ability of iridial melanin to generate superoxide anion and hydrogen peroxide and the effects of the melanin on peroxidation of lipids by employing EPR oximetry, EPR-spin trapping and oxidase electrode, respectively. Our data show that the human iris contains predominantly eumelanin. The content of iridial melanin is about 40% higher in brown eyes, compared to blue eyes, and shows little variation with donors age. Although aerobic photoreactivity of eumelanin in low, its efficiency to photogenerate superoxide anion and hydrogen peroxide increases in the presence of ascorbate. We have demonstrated that iridial melanin can quench electronically excited states of photosensitizing dye molecules, sequester reactive metal ions and inhibit lipid peroxidation. Supported in part by NIH and State Committee for Scientific Research.
IL255
Melanin production, photoprotection and photodamage in in vitro cell culture models
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In vitro skin cell cultures offer a straightforward model for studying many aspects related to skin photobiology: e.g. the role of melanin production in photoprotection and photosensitization. When complemented with reliable biochemical and analytical methods, data from experiments in cell culture models can significantly contribute and expand our contemporary knowledge of photobiology. New experiments were aimed at prevention of oxidative damage in skin melanocyte and fibroblast cultures. Various antioxidant combinations were tested for their potential to prevent UV induced lipid peroxidation and oxidative DNA damage. For this purpose lipid peroxidation was measured by detection of malondialdehyde using HPLC. The comet assay was used to detect DNA damage. Endogeneous production of reactive oxygen species was measured by using dihydrorhodamine (DHR) 123 and FACS analysis. Preincubation of cells with antioxidant mixtures before irradiation resulted in clear reduction in lipid peroxidation. DNA damage was reduced in melanocytes pretreated with vit E and C in combination with carotenoids. DHR 123 was useful to show differences in endogeneous production of ROS in melanocytes of various origin and the effects of antioxidants. The results indicate that antioxidant treatments can protect against UV induced oxidative damage and may have important consequences in the process of photoaging.

IL256
The Structural and Reactivity of Natural Melanins
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The structure of melanin extracted from the ink sac of the cuttlefish Sepia officinalis was examined for different methods of isolation and purification of the pigment. Scanning electron microscopy (SEM) images of Sepia eumelanin prepared by different procedures establish that the dominant constituents of Sepia melanin are ~150 nm spherical granules, and the multi-μm-sized aggregates reported by previous workers are generated by their sample preparation. Different drying techniques used generate different aggregation morphologies, particularly the surface area is affected. Brunauer-Emmett-Teller (BET) measurements reveal that Sepia eumelanin prepared by spray-drying, freeze-drying and CO2-supercritical drying has a surface area of 14.3 m²g⁻¹, 21.5 m²g⁻¹, and 37.5 m²g⁻¹, respectively. Ultra-high resolution SEM and atomic force microscopy (AFM) images show that the surface of the granules is not smooth and the granules are comprised of smaller constituents. De-aggregation of the granules by sonication and ultrafiltration reveal a range of structures depending on the pore size of the membrane used. The implications of these results on quantifying photochemical properties and kinetic reaction rate constants of melanin are discussed.
Why Aquatic Bacterial Isolates Show Different Sensitivity to UV Radiation?

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Pelagic bacteria from marine and freshwater systems constitute an important carbon pool available to protists and metazoans. Several studies have shown that solar ultraviolet radiation (UVR) negatively affects activity and growth of aquatic heterotrophic bacteria. Most of the studies, however, have assessed the impact of UVR on whole bacterial communities. Nevertheless, there is evidence for interspecific differences in UV-sensitivity among bacterial isolates, but the reason for it is not known. In this study, we addressed the question how single-cell characteristics and environmental conditions may alter bacterial sensitivity to UVR. For this, we cultivated several bacterial strains isolated from an alpine lake under low nutrient concentrations. Four isolates including two pigmented and two non-pigmented strains were grown at 6 and 25 °C for several months and tested for their UV-sensitivity under artificial UVR. Sensitivity of bacteria was assessed by measuring protein production by 14C-Leucine incorporation, number of viable cells by cfus on agar plates, abundance of active bacteria by FIB-FDA, and the proportion of membrane-compromised cells by staining with PI and SYTO 13 and detection by flow-cytometry. First results of these experiments indicate that pigmentation in aquatic bacteria may be responsible for interspecific differences in UV sensitivity.

Influence of UV radiation on photomovement parameters of Dunaliella viridis and Dunaliella salina.

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The effect of ultraviolet (UV) radiation on photomovement parameters of two species of green algae of genus Dunaliella (D. salina and D. viridis) was investigated. The velocity of movement doesn’t depend on the intensity, wavelength and duration of irradiation in contrast to phototropaxis. This fact testifies to possible difference in mechanisms which govern these parameters of movement. It was elicited for the first time the conversion of positive phototropaxis into negative one under UV irradiation with further inhibition of phototropaxis induced by increased duration of irradiation. The most inhibitory effect on phototropaxis was provoked by UV-radiation at 248-280 nm. Two species of Dunaliella can be used as biological dosimeters of natural ultraviolet radiation.

Influence of divalent cations on violaxanthin de-epoxidase activity

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Violaxanthin de-epoxidase (VDE) is one of the two enzymes involved in the xanthophyll cycle, which is a mechanism for short-term adaptation of plants to varying light intensities. Upon high light, VDE converts the carotenoid violaxanthin to zeaxanthin, a molecule that is involved in non-photochemical quenching of excess energy. The regulation of VDE activity with divalent cations has been studied in thylakoids and on isolated enzyme. MgCl2 was found to inhibit VDE activity already at a concentration of 10 μM while EDTA stimulated enzymatic activity. Treatment of VDE with EDTA also shifted the pH-optima to slightly higher values. Inactivation of VDE showed to be a slow process, even at high MgCl2 concentrations. However, after 10 min of incubation at pH 5.2 and 26 °C, VDE activity was fully inhibited. Treatment of isolated enzyme with CaCl2 also inhibited activity. We suggest that flow of Mg2+ from the lumen to the stroma under strong light contribute to the regulation of VDE activity in vivo.

Measurement of erythema-effective irradiance and determination of skin type as conditions for a responsible use of solaria

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For safety reasons, it is recommended that the erythemal radiant exposure of each session is distinctly below the personal MED of each solarium user, e.g., the MED is related to 1%. Therefore, for calculating the recommended exposure time it is necessary to know about the erythema-effective irradiance Ee and the personal MED of the user. The measurement device MSS 400 presented here measures both. 1. Erythema-effective irradiance The erythema-effective irradiance is measured by using a detector whose spectral sensitivity is adjusted to the action spectrum for the erythema (see CIE and DIN 5031-10). 2. Personal MED The measurement device MSS 400 measures the spectral diffuse reflection of the skin by using four selected wavelengths, and determines the skin type from it. From the skin type the personal MED of the solarium user is calculated following the guidelines of the national standards or safety regulations. The software of the measurement device stores the measurement values for the erythema-effective irradiance Ee for a maximum of 10 solaria. The values of the user’s skin types are also stored so that the personal exposure times for each solarium user are determined. The software also considers the increase of the exposure times as reaction of the skin.

Analysis of the genotoxic effect of combined UVB and UVA irradiation of human keratinocytes: Evaluation of the protective capacity of a sunscreen

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Ultraviolet rays are involved in the induction of skin cancer. UVB radiation is absorbed directly by the DNA, causing the formation of bipyrimidine photoproducts such as cyclobutane pyrimidine dimers (CPD) and pyrimidine (6–4) photoproducts. Although UVA radiation is only weakly absorbed by DNA, it is known that UVA also generates bipyrimidine photoproducts. The contribution of UVA to the UVB-induced DNA damage could increase the mutagenicity. Secondly, the UV-generated lesions were specifically analysed by HPLC/MS-MS. The distribution of CPD and (6–4)PP is modified after UVB + UVA irradiation compared to simple irradiation with UVB or UVA alone. Moreover, UVB + UVA induces the appearance of the valence isomers of (6–4)PP, the Dilara photoproducts. The contribution of UVA to the UBV-induced DNA damage could increase the mutagenicity.
The National Science Foundation is gratefully acknowledged.

Irradiation of equimolar to increasing amounts of corresponding cyclobutane dimers of thymine dimer is converted into the corresponding m5C-T mixed dimer upon incubation. The trans-syn cyclobutane reaction occurs considerably faster at 310 K. The cyclobutane dimers of 5-methylcytosine and their deamination of UV-B exposure the amount of TT-dimers was identical in heat treated and 24 hrs after UV-B irradiation when high levels of CPDs are still present in the genome. The present study was investigated whether heat shock interferes with the formation and repair of UV-B-induced photoproducts (thymine (TT)-dimers). This DNA damage appears shortly after UV-exposure and is consequently repaired. We compared the formation of TT-dimers after UV-B exposure in heat treated and control normal human keratinocytes, melanocytes, and fibroblasts employing chain reaction using short and long segments of phage T7 DNA and in isolated T7 DNA and in isolated T7 DNA and in isolated T7 DNA. A major observation was the removal of UV-B-induced TT-dimers. In contrast, our results might indicate an alteration in DNA structure that can result in increased induction of photoproducts.

These observations emphasize the role of CPDs in the mutagenic effects of UV-B. The cyclobutane dimers of 5-methylcytosine and their deamination sequences in cultured human dermal fibroblasts. A major observation was that the rate of repair of TT and TC photoproducts significantly decreased with increasing UVB dose. Therefore, a relatively large amount of CPDs remained 48 h after irradiation, whereas (6-4) PP were efficiently repaired within less than 24 h, irrespective of the dose. Since the overall applied doses (800 500 J m-2) were chosen to induce moderate lethality, fibroblasts could recover their proliferation capacities following transitory cycle arrest, as shown by 5-bromo-2′-deoxyuridine incorporation and flow cytometry analyses. As a result, UVB-irradiated cells were found to normally proliferate 48 h after irradiation when high levels of CPDs are still present in the genome.

In many plants the role of photoreceptors is played by a flavine. The action spectra of living organisms are more similar to those of FMN dimers rather than to the monomer absorption spectra. Electronic absorption spectra and polarized absorption spectra of flavin mononucleotide (FMN) in polynuclear alcohol films (PVA) were measured over the concentrations ranging from 6.9·10-10 M to 6.8·10-4 M. The absorption spectra of high concentrated FMN molecules in PVA matrices (c=10-10 M) occurred to be almost independent of concentration and they nearly overlapped with the dimer spectrum.

Influence of heat shock on DNA-repair after UV-B


Phage T7 can be used as a biological dosimeter, its reading, the biologically effective dose (BED) is proportional to the inactivation rate |ln (n/n o)|. For the measurement of DNA damage in phase T7 dosimeter a quantitative polymerase chain reaction (QPCR) methodology have been developed using a 55S bp and a 3826 bp fragments of phase T7 DNA. Both optimized reactions are so robust, that equally good amplification was obtained when intact phage T7 was used in the reaction mixture. In the biologically relevant dose range a good correlation was obtained between the BED of the phage T7 dosimeter and the amount of UV photoproducts determined by QPCR with both fragmentss under the effect of various UV sources. A significant decrease in the yield of photoproducts was detected by QPCR in isolated T7 DNA and in heated phage compared to intraphage DNA with all irradiation sources. As the yield of photoproducts was the same in B, C and A conformational states of T7 DNA, a possible explanation for modulation of photoproduct frequency in intraphage T7 DNA is that the presence of bound phage proteins induces an alteration in DNA structure that can result in an increased induction of photoproducts.

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The photochemical reactions of 5-methylcytosine (mC) have been studied in frozen 10 mM aqueous NaCl solution at dry ice temperature. For these studies, low pressure lamps emitting mainly UVB radiation (Spectronics BLE-1T158) were used. We have found that three cyclobutane dimers are produced, namely the cis-anti, the cis-syn and the trans-syn forms. While the cis-anti and the trans-syn cyclobutane dimers are relatively stable towards deamination upon standing at 277 K in solution, the cis-syn isomer is slowly converted into the corresponding cis-syn mC-methylene (T) mixed dimer; this latter reaction occurs considerably faster at 310 K. The trans-syn cyclobutane dimer is converted into the corresponding mC-T mixed dimer upon incubation. While the cis-anti and cis-anTi mixed dimer when incubated at 310 K. Longer incubation times lead to increasing amounts of corresponding cyclobutane dimers of thymine being present in the various incubated solutions. Irradiation of equimolar mixtures of T (1 mM) and mC (1 mM) under similar conditions yields each of the mC cyclobutane dimers, as well as significant amounts of cis-anti, cis-syn and trans-syn mC-T mixed cyclobutane dimers. Research support from the National Science Foundation is gratefully acknowledged.
P013

EPR study of photoinduced changes in natural humic substances.
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Humic substances (HS) were obtained from walnut hulls Juglans regia. An EPR signal was observed for powder sample with spin density of 6.10^20 spin / g. Irradiation with 150 W xenon lamp leads to 30% increase in amplitude that was constant during 20 min of irradiation. After the irradiation was switched off the amplitude of the EPR signal returned to the initial value.

Aqueous samples of HS at 2 mg/ml concentration also have shown EPR signal. The samples were irradiated with visible light with \( \lambda > 390 \) nm, \( \lambda < 340 \) nm, \( \lambda > 280 \) nm and with full UV light \( \lambda > 200 \) nm. The EPR signal amplitude increases as the energy of irradiation increases. Kinetic study of EPR signal amplitude of irradiated HS exhibit different behavior depending on the atmosphere of the sample. The samples were maintained in air or were purged with argon.

The obtained results and the observed differences between EPR signals of the samples under different atmosphere indicate on the two different radical mechanisms which influence the formation and the degradation of HS during solar activity.

This work was supported by grant 3 PO49 016 22 from the State Committee for Scientific Research, Poland

P015

Polychromatic action spectrum for photosynthesis inhibition in Dunaliella tertiolecta after long-term, cyclic exposure to UV-B radiation
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With the aim to study how the increase of solar ultraviolet radiation affects phytoplankton photosynthesis, we have treated cultures of the green microalgae Dunaliella tertiolecta with long-term, cyclic exposure to polychromatic radiation, simulating natural irradiation conditions.

Previous experiments had shown that short time (30-60 min) exposure to UV-B radiation caused a temporary photosynthesis inhibition. In the present work samples were kept over a week under a pair of "daylight" fluorescent lamps, with a light/dark cycle of 15/9 hours. On the first 5 days, during the 5 central hours of the light period, UV-B radiation was added with different spectral bandwidths and intensities; on the last two days the cultures were left under visible light only, to test for recovery processes. Photosynthesis was evaluated by measuring the optimal quantum yield \( F_v/F_m \) by means of a PAM fluorometer.

At the lowest UV irradiance (0.5 W/m^2) we observed strong inhibition only in the case of the spectral distribution obtained with a 290 nm cut-off filter, whereas at the highest irradiance (1.0 W/m^2) strong inhibition was observed in the case of the spectral distributions with 290, 300 and 310 nm cut-off filters. In all these cases no recovery was observed.

P016

Dependence of PAR spectral distribution on soil covering and altitude in Chianti Rufina (Firenze, Italy) vineyards
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This work reports on spectral irradiance measurements in two vineyards at different altitudes (Pomino, 70 m a.s.l. and Poggio a Remole, 700 m a.s.l.) a month before harvesting, to evaluate how the spectral distribution of the solar radiation reaching the leaves is affected by soil covering and altitude.

In the same optimal meteorological conditions, the lower vineyard receives 5% and 6% more of total PAR radiation and blue radiation, respectively, whereas the Red/FarRed ratio is the same at both altitudes. The difference in irradiance in the blue region could also be more significant, due to the higher level of tropospheric aerosols and haze in the Remole vineyard.

Soil spectral albedo was also measured in the Pomino vineyard, where different soil coverings were present (natural tilled soil, covered crop soil and artificially covered soil with the reflective material Vitexol). The artificial covering was very effective in reflecting blue radiation and significantly effective for the red one (8 and 2 times higher than natural soil, respectively). The tilled soil reflected red radiation 40% more than the covered crop soil and the Red/FarRed ratio was 20% higher, notwithstanding the chromatic similarity of the two surfaces.

P017

Comparison of erythemal weighted irradiance from YES UVB Broadband and SL501A Broadband Radiometers under all sky conditions
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UVB Broadband Radiometers that measure erythemally weighted irradiance are widely used by many research programs because of their low cost and ease of use. Because currently available UVB radiometers’ spectral response functions do not exactly match the erythemal action spectrum, calibration factors as a function of total ozone and solar zenith angle are necessary for accurate results. This work presents comparisons between YES UVB radiometers and SL501A UVB radiometers over a six year period under all sky conditions. These UVB radiometers are also compared to erythemally weighted irradiance from a precision UV spectroradiometer. The UVB radiometers and UV scanning spectroradiometer used for this study have been calibrated on a yearly basis by the U.S. Central UV Calibration Facility (CUCF) at the National Oceanic and Atmospheric Administration. The CUCF has three YES UV-B broadband radiometers and three SL501A UVB broadband radiometers that operate in the field at the CUCF’s Table Mountain Test Facility (TMTF). These six broadband radiometers are run simultaneously with the USDA UV Monitoring Programs’ reference U111 Spectroradiometer developed by Atmospheric Science Research Center (ASRC) at SUNY.

P018

Response of pigments in sea ice algae to ultraviolet radiation exposure - time series studies
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Antarctica’s ozone hole is known to be largest during the early spring, often coinciding with the break up and retreat of the sea ice. At that time, ice algae populations are potentially exposed to increased ultraviolet radiation (UVR), as well as photosynthetically available radiation (PAR). Between 26 September and 18 October of 2001, on board the RVIB Nathaniel B. Palmer, four experiments were carried out to determine the response over time of photosynthetic and photoprotective pigments in sea ice algae to UVR. Samples were collected for nutrients, and both a total (>0.7 μm) and a <5 μm fraction were collected for HPLC pigment analysis. Results from the first experiment show that both photosynthetic and photoprotective pigments (chlorophyll a, fucoxanthin, 19'-hexanoyloxyfucoxanthin, diadinoxanthin and diatoxanthin) in the total fraction were increasingly inhibited over the course of the exposure. Inhibition of pigments = (pigment_{initial} - pigment_{final})/pigment_{initial}. The c50 fraction inhibition did not increase in time, and fucoxanthin showed decreasing inhibition in the course of the 48 hour experiment. Results from this first experiment show that UVR damage to pigments does not show repair to counteract net inhibition, but that diatoms smaller than 5μ appear to be selectively more resistant to UVR.
Role of phospholipase D in the processes of photo- and scotomorphogenesis in plants

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It was studied the role of phospholipase D (PLD) in the processes of photo- and scotomorphogenesis in plants.

We found that PLD activity in dark- and light-grown oat seedlings differed dramatically. The illumination of etiolated seedlings with white or red (R) light decreased PLD activity. In contrast, the activity of enzyme in green seedlings was increased after their transfer for a long period to darkness. It was also shown that the light conditions affected the intracellular localization of PLD activity. It can be supposed that photoreceptor phytochrome is involved in photomodulation of PLD activity. There is evidence that R action is mediated by G-proteins with involvement of phosphoinositide cycle. Light modulation of PLD activity depended on soluble sugar level. The addition of glucose or sucrose to etiolated seedlings suppressed PLD activity similar to the light illumination. On the other hand, sugars prevented the dark-induced increase in PLD activity in green seedlings. Application of a photosynthesis inhibitor, diuron, caused the activation of PLD under illumination.

Thus, the obtained results suggest that PLD activity in oat seedlings depends on light conditions of growth and carbohydrate metabolism.

Membrane-bound guanylate cyclase activity in cells of light and dark-grown Avena sativa seedlings

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cGMP is supposed to be a putative intermediate involved in transmission of signal from plant photoreceptors to target elements in the promoters of light-regulated genes. The structural aspects, localization and functional role of guanylyl cyclase (GC) responsible for cGMP synthesis in plant cells are not sufficiently investigated and plant genes encoding GC are not yet sequenced and cloned.

The highest GC activity in subcellular fractions of oat seedlings was found in plasma membranes. The GC activity depended on light conditions during plant growth. It appears to be lower in tissues of dark-grown plants than in light-grown seedlings. GC activity was 4-fold stimulated by red light addressed to phytochrome and far-red light irradiation removed the stimulating effect. Thus, cGMP content in plant cell is controlled by light with the involvement of phytochrome addressing its action to GC.

GTPS was found to suppress both GC activity and red-light stimulating effect. On the contrary, GTPs had an opposite influence. Thus, membrane-bound GC in plant cell is likely to be controlled by G-proteins and G-proteins are involved in regulation of GC activity by light.

He:Ne laser stimulates the activation of Telomerase in Circulating human lymphocytes

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Telomeric DNA protects chromosomes from recombination events and its length serves as a mitotic clock that triggers exit from the cell cycle when telomeres become too short. Telomerase is the enzyme involved in telomere elongation, one of the events that permit unlimited cell proliferation. Telomerase activity is expressed selectively in germ-line and malignant tumor cells but not in most normal human somatic cells. As a notable exception, telomerase is expressed in human lymphocytes during development, differentiation, and activation. Variations in telomerase activity were quantified in human non-stimulated lymphocytes after He:Ne laser irradiation A modified telomeric repeat amplification protocol (TRAP). Telomerase activity increased after irradiation of between 0 and 10 J/cm2 in a dose-dependent manner in bufft coat irradiated lymphocytes, reaching a maximum at 10 J/cm2. The increase in telomerase activity was nearly maximum 8 h after irradiation, the peak being observed at around 24 h. Meanwhile, purified lymphocytes did not exhibit telomerase activity compared to non-irradiated. The activation of telomerase observed after He:Ne laser irradiation may suggest the involvement of telomerase in DNA repair and chromosome healing.
The influence of LED irradiation at different wavelengths on functional biological effects of light irradiation.

The primary effects of light can be observed in changes in the function of the endothelium. Endothelial cells produce and/or release a variety of substances that modulate vascular tone as well as growth, coagulation, and platelet function, and the release of circulating hormones. Some of these are nitric oxide (NO), NO synthase, endothelin-derived hyperpolarizing factor, and prostacyclin (prostaglandin I$_2$, PGI$_2$), prostaglandins, endothelin, and angiotensin II.

The presentation discusses the biochemical construct of NO synthase (source of NO) and light of different wavelengths which can be absorbed by the different components on NO synthase and modulate its function. Several isoforms of the enzyme occur in endothelial cells, as well as in platelets, macrophages, vascular smooth muscle cells, and the brain.

Relaxation of smooth muscle cells by NO is associated with activation of soluble guanylate cyclase (GC) and an increase in intracellular cyclic 3′,5′-guanosine monophosphate (cGMP) in vascular smooth muscle. Increased levels of cGMP in platelets are associated with reduced adhesion and aggregation. Endothelium-derived NO is formed from L-arginine by oxidation of its guanidine-nine terminal, also regulated by light. NO synthase reacts to light somewhat in the same pattern as cytotoxic P450.

Changes of production of some biologically active substances by the endothelium under light irradiation (biochemical aspects).

The aim of the study is to compare the effects of LED light irradiation of different wavelengths on induced blood platelet aggregation. LED clusters were used as sources of light. The study was conducted on Wistar rats. The level of induced blood platelet aggregation was measured by impedance aggregometry. ADP and adrenalin were used as aggregating agents. The blood samples of test groups were exposed to LED irradiation (430, 565, 595, 660 and 880 nm) prior to aggregation induction. The control group blood was tested without light irradiation. In all test groups inhibition of aggregation and 880 nm) prior to aggregation induction. The control group blood was tested without light irradiation. In all test groups inhibition of aggregation and 880 nm) prior to aggregation induction. The control group blood was tested without light irradiation. In all test groups inhibition of aggregation was recorded.

The strongest inhibition of aggregation in case of a single dose of irradiation (0.045 J/cm²) was detected after blue and red light exposure (37% and 45% accordingly, p<0.05). It was also noted, that in case of red and blue light, inhibition of aggregation became stronger according to increase of irradiation dose, while in case of green, yellow and IR light the weakest dose caused the strongest inhibition.

Presented data confirmed with the results of Brill G.E. (1998) showing inhibition effect of red laser light irradiation on blood platelets aggregation due to activation of guanulatecycase and increase of cGMP amount in irradiated platelets.

Modifying influence of low level laser irradiation on the relationships in endothelial cell – blood platelet system.

The expression of P-selectin was detected as a sign of activation of adhesive function of the cells after 20 min of laser irradiation. Higher adhesion rate of intact platelets to irradiated endothelial cells was revealed. So, activation of endothelial cells and higher ability of adhesion of platelets were results of laser irradiation of endothelial cells.

Platelet response to laser irradiation was completely different. Inhibition of platelet activation by TRAP due to decrease of expression of P-selectin and weaker ability of binding fibrinogen by GPIIbIII membrane receptor were detected. Inhibition of aggregation induced by different inducers (ADP, ristocetin, adrenalin, collagen) and inhibition of adhesion on extracellular matrix, collagen covered surfaces as well as intact vascular endothelium was detected. The inhibitory effect of laser irradiation on platelets was mediated by phototocislation of guanulatecycase, higher amount of cGMP and inhibition of intracellular reactions with proteinase C participation.

In conclusion, the different way of reacting of endothelial cells and platelets on laser irradiation is an important factor, supporting circulatory haemostasis during intravenous laser blood irradiation.

Biochemical effects of light irradiation and kinins.

The primary changes in cells induced by light are followed by biochemical reactions which continue in darkness. These reactions are associated with the changes of cellular homeostasis, specifically the cellular redox state. The change of the pH is one of the regulating factors for kinin production. Kinins are potent vasodilator peptides generated in blood and tissues. Naturally occurring kinins and their active metabolites exert various biologic actions. Kinins are among the most potent activators of the arachidonic acid cascade, and it promotes the release of prostaglandins and prostacyclin. Kinins also stimulate the release of histamine and 5-hydroxytryptamine from mast cells. They modulate the motility and the function of leukocytes, macrophages, fibroblasts, and other cells. Kinins are also implicated in the pathogenesis of inflammation, tissue reactions to injury, and tissue repair.

Kinins release a potent endogenous vasodilator from the endothelium that reduces the arterial smooth muscle tone, increasing the blood flow to organs and reducing the systemic blood pressure. Kinins increase capillary permeability, which can be associated with the appearance of superpositional continuous quasi-stable states and vibra-tional-rotational continuum of energy. The primary effect of LAS is coherent vibration-rotational excitation of enzymes with subsequent conventional reconstruction and activation. The mathematical model of the coherent mechanism has been developed. The results of calculations conform to experimental data. The schemes of experimental investigations, directly corroborating the mechanism of LAS, are proposed. They allow to define new parameters of biocompounds which represent a certain scientific interest.

The primary act wave mechanism of the laser stimulation of the biomolecules.

The wave or coherent mechanism of interaction between light and biomolecules has been developed. This mechanism is based on experimental results of laser biostimulation (LBS) and is able to explain the effects observed. The model of the coherent primary mechanism of interaction is based on the (fixed in many experiments) dependence of LBS efficiency on degree of light coherence, and so-called spectra of LBS, in particular, presence of effects of LBS and therapy within red-IR spectrum range, where majority of biomole- cules don't absorb photons. The targets of laser radiation in this mechanism are enzymes. Owing to the enormous number of atoms (~10^4) in such macromolecules different types of vibration and rotation interact and the overlaps of wave functions of separate states occur, that then leads to appearance of superpositional continuous quasi-stable states and vibra-tional-rotational continuum of energy. The primary act of LBS is coherent vibration-rotational excitation of enzymes with subsequent conventional reconstruc-tion and activation. The mathematical model of the coherent mechanism has been developed. The results of calculations conform to experimental data. The schemes of experimental investigations, directly corroborating the mechanism of LAS, are proposed. They allow to define new parameters of biomolecules which represent a certain scientific interest.

The influence of LED irradiation at different wavelengths on functional activity of blood platelets.

The influence of LED irradiation at different wavelengths on functional activity of blood platelets was investigated. HUVEC endothelial cell culture was irradiated by red laser.

Some effects and mechanisms of laser biostimulation.
P031 Some Effects and Mechanisms of Laser Biostimulation
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Several studies were conducted on the enhanced healing of cut and repaired rabbit Achilles tendons using He:Ne and 904 nm IR lasers of 4 J/cm2 fluency 3 times a week for 3 weeks. Significant enhancement of healing of crushed rabbit median nerve was observed after applying He:Ne and IR 904 using 12 J/cm2 fluencies three times weekly for one month. IR laser of 810 nm of 4 J/cm2 fluency applied thrice weekly for three weeks, enhanced bone healing after fracture fixation of rabbit mandible fracture, as shown by radiography and histopathological assessment. In 40 patients applying He:Ne and 904 IR lasers at 1-4 J/cm2 2-3 times a week to chronic leg ulcers, as well as in serum and tissue fibroblast growth factor and epidermal growth factor and DNA after laser. Irradiation human lymphocytes with various fluencies of He:Ne laser induced mitogenesis without causing any genotoxic effects. These results may shade some light over the mechanism of laser biostimulation. Still there is need to standardize light dosage to guard against controversial results.

P032 Mechanisms of anti-inflammatory, immunomodulatory and wound-healing effects of the polychromatic (visible and infrared) light: summary of the 3-year-long studies
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Effects of visible and infrared light on human health are poorly investigated. Taking into consideration a sufficiently deep penetration of these radiations into human skin and possibility of the transcutanous blood photomodification, laboratory studies more than 25 blood parameters in 500 volunteers after their exposures to polychromatic visible + infrared polarized (VIP) light of the Swiss phototherapeutic device “Bioptron” (480-3400 nm, 95 % of polarization, 12 J/cm2). As soon as at 0.5 h changes in circulating blood have been established: modification of the structural state of cell membranes, improvement of rheological and transport properties of erythrocytes, decrease of platelet aggregation, activation of leukocytes phagocytosis, modulation of cytoxic activity of NK cells and lymphocyte DNA synthesis, a decrease of inflammatory cytokine levels, an increase of the content of anti-inflammatory cytokines and growth factors, an enhancement of plasma capacity to stimulate proliferation of skin cells in vitro, etc. Similar changes are also recorded at irradiation of blood in vitro as well as at modelling the events in vascular bed of VIP-exposed volunteers by mixing the irradiated and non-irradiated autologous blood samples (1:10). All the above effects are most likely due to the light-initiated, blood cell-mediated cascade of the reactive oxygen species.

P033 Systemic mechanisms of wound healing and prevention of scar formation by exposures of human body to polychromatic (480-3400 nm) light
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Visible and infrared light from laser and non-laser sources stimulates wound healing and prevents scar formation. In this randomized, placebo-controlled, double blind trial we demonstrated that soon after a single and repeated exposures of volunteers’ body surface (254 cm2) to polychromatic visible-infrared polarized (VIP) light of Swiss phototherapeutic device “Bioptron” (480-3400 nm, 95 % of polarization, 12 J/cm2) soluble factors arear in blood, able to stimulate under condition in vitro proliferation of the human keratinocytes and endothelial cells to the 3-5 times greater degree and with the higher frequency than that of fibroblasts. The similar difference is revealed on testing plasma after VIP-irradiation of blood in vitro and after modeling the events in vascular bed of VIP-exposed volunteers by mixing the irradiated and non-irradiated autologous blood samples at a volume ratio 1:10. Hence, after light treatment the human blood is able “to translate” the light-induced changes to much higher volume of autologous blood. The results obtained suggest the differential enhancement by light of the growth promoting activity of the entire circulating blood for some target cells, which might be responsible for acceleration of wound granulation and epithelization as well as for prevention of hyperproduction of connective tissue cells.

P034 Analysis Of Staple Fibres Colagenas And Macrophages Through The Morfometria Computerized In Cutaneous Wounds Of Submitted Rats Irradiation Of Laser Hene
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The present work had as objective to compare the results of the effect of the laser therapy in the incisional regeneration of cutaneous wounds. Through the morphometric analysis. This analysis in supplied to the percentage of collagen staple fibres for color density and the number to them of macrophages. We use in this study 48 Wistar rats, males, who had been submitted to an injury in the back. For the treatment of Group I we use the HeNe Laser with dosage of 4 J/cm2 for 36 s. the removed samples, respectively, in 3º day, 7º and 14 days the injury had been H.E., Tricribó de Masson and submitted the immunoistoquimica for monoclonal antibody HAM 56. The morphometric analysis was carried through by software IMAGELAB. The results of the samples had been dealt with statistic by the analysis variance ANOVA, and test of Tukey getting itself in both, p<0.05. One concludes that: The application of the laser radiation develops the cicatricial process forming a collagen staple fibre net better elaborated of that in animals not submitted to the same treatment; It has a satisfactory reply of the macrophages when submitted to the laser radiation.

P035 Combination of textile and cosmetic sun protection
H. Tronnier, B. Hölzner, U. Heinrich; DERMATOKONNIK Witten, Germany.
The UV-radiation of human skin by sun irradiation can be decreased by photorefrainence, sun protection products (SPF) and wearing appropriate clothes. For the tests an in-vitro method was used. This procedure is in accordance with the international norms for testing sun protection efficacy for textiles (UPF). For sun protection products this method has also proved for UVA and UVB. For the study a sun care product was used and 4 textile samples (silk, chiffon, cotton). The measuring device which was used is suitable for determining of the sun protection factors and the stability of sun protection products and textiles. Fabrics used for summer and bathing clothes only offer minimal to low sun protection. An improvement by integrated light filters or filters which are applied during the washing process seems feasible. An advantage of the textiles is their UVB stability. Only the SPF showed a decrease in efficacy under UV irradiation. In the UVA the protection factors for textiles are generally a little bit higher, those for the SPF, however, are lower than in the UVB. Informing the consumers about possible advantages of a combination of chemical and textile protection is important. The protective effect might be maybe mentioned on the label of the clothing.

P036 Intense pulsed light for photo-rejuvenation and freckles of Middle Eastern skin
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Facial ageing is a gradual process which could be due to intrinsic and extrinsic causes. It ultimately results in the appearance of gravity-induced tissue ptosis, wrinkles, epidermal and dermal atrophy, dryness, senile Lentigo, flushing, telangiectasia, and enlarged pores. Moreover, Freckles are frequently seen on the face and other sun-exposed areas, it is characterized with increased melanin in the epidermis. Intense Pulse Light (IPL) is the latest technology for selective photo-thermolysis as a non-ablative photo-rejuvenation. A thirty four Egyptian patients of age ranging between 35-70 years old with skin type ranging between (II-V) with or without freckles were treated using IPL in a 3 week sessions for 3-5 sessions. Irradiation wavelength was controlled using cut-off filters ranging from 550 to 580 nm, with a fluence of 25-35 J/cm2. A significant improvement was demonstrated after 6 months compared with the baseline. CONCLUSION: IPL is an effective and safe treatment for fine wrinkles, facial freckles, telangiectasia as well as post-inflammatory hyperpigmentation with relatively a few adverse effects and high satisfaction level.
evaluation of phototherapeutic dose in PDT depends on administered dose of photosensitizer, the incident light dose and time interval between administration of photosensitizer and light. Many of interdependent dosimetry factors affect the estimation of the effective phototherapeutic dose. Photobleaching could be used as dosimetry index, since it reflects those dosimetry factors, which could not be measured directly. The ratio of the initial fluorescence intensity to the intensity measured during light treatment could be used as a relevant criterion of tissue response; e.g. tumour necrosis. C57Bl/129Sv mice bearing A22 tumours in the right hip and sensitized with chlorin e6 (10 mg/kg) were used in the experiment. Laser irradiation (I = 355 nm) was performed 4 hours after administration of chlorin e6 (fluence 200 J/cm2). Photobleaching measurements in vivo and scanning of tumour surface ex vivo were performed using blue LED light (λ = 410 nm). The number of necrotic and intact tumour cells was counted ex vivo by microscopy analysis. Tumour necrosis depth was tested by staining with methylene blue. Chlorin e6 fluorescence variation in tumour area was related to staining and microscopy analysis data, as well as light intensity distribution within the tissue.

The role of these neurones in the some modulation of central nervous system and play the role of the adrenergetical mediators. Cateholamines, in particular, nor-adrenaline and dopamine are arised in the mechanism of the muscles cells, which is dependent upon the energy receiving and enfeeblement, connected with the hyper-polarization and electrical peace.

Besides, a possible new mechanism of laser-information phenomenon and understand in definite degree a role of stochastic features of dopaminergical neurones. The role of these neurones in the some modulation of central nervous system and play the role of the adrenergetical mediators. Cateholamines, in particular, nor-adrenaline and dopamine are arised in the mechanism of the muscles cells, which is dependent upon the energy receiving and enfeeblement, connected with the hyper-polarization and electrical peace.

In our paper at first we propose to consider a problem of the interaction between the biological cell and ionizing radiation field within neural networks approach. The finite temperature fluctuations (correlation functions) are also calculated. The «zwitterion» appears to be strongly favoured with respect to the neutral molecule. In this situation one can wait for the PG phenomenal biocalalytic and photochemical activity. Using the selective two-stepped (IR+UV) and multi-photon (IR) laser field action method for biological molecules in the solution it is connected with known difficulties. We carried out a general theoretical consideration of using the IR+UV two-stepped photo-excitement method with excitation of relatively isolated vibrations which have not strong Fermi resonances with other vibrations for the photochemistry of PG in water solution. Besides, it is studied a possibility of selective excitation of vibrational levels by means of the Raman process in a field of the two-frequency visible laser radiations.

The molecule geometry's for the solute together with additional data (computed net charge and class for each atom etc.) are taken and compiled from 1-3. The water-water interaction potential was obtained by Matsouka et al. [c.f. (2)] from configuration interaction calculations. The potential was used in the MC calculation of the water and found to reproduce the experimental as for energy and structural properties. The bio-molecule-water interaction potential was obtained in the SCF-ICAO-MO approximation and fitted with an analytical function. Calculations were carried out at T=300K and all molecules were treated as rigid. Results for potential energies are following (in kJ mol-1): Water-water (neutral molecule) -28.8±0.5 and («zwitterion») -28.5±0.5; PG-water (neutral molecule) -63.6±2.5 and («zwitterion») -37.6±15;0; The MC result for bulk water (with the same interaction potential) is 35.6±0.6 kJ mol-1 [2]. The structural characteristics (radial distribution and orientational correlation functions) are also calculated. The «zwitterion» appears to be strongly favoured with respect to the neutral molecule.

P039 Neural networks models and general strategies in complex studying the harmanic action mechanism: Stochastic resonance phenomenon and laser - informative action
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Paper is devoted to studying the biophysical and biological realization of the neural networks. We start from the problem of the harmon of own action mechanisms investigation. There are in details considered the main mechanisms of the harmonic action such as the cell membranes porosity changing, the direct ferment activation, the ferment synthesis on the ryhobosomes stimulation, the action by means of the adenylylacylasa system (a generalization of the second theory, the induction of the ferments synthesis on the cell nucleus. Considered mechanisms are a basis for the biophysical realization of neural network. Problem of the action mechanism study of cateholamines on the muscles is also considered on the basis of the neural networks modelling. We modelled the dependence of the cateholamines action mechanism upon the influence of the muscles cells polarization, in particular, the contraction because of the depolarization and action potential appearance and the influence on the hyper-polarization and electrical peace. Last phenomena are defined in known degree by the sodium transport mechanism of the muscles cells, which is dependent upon the energy receiving and the adenilacylasa system particpance, cells phosphorhiaz concentration. Two cateholamines, in particular, nor-adrenaline and dopamine are arised in the central nervous system and play the role of the adrenergetical mediators. Because of the low activity of the dopamine-bethadryhrolozla it is possible a situation when the domapine biosynthesis is stoped and, as result, the non-adrenaline is not arised. This is connected with the availability of the dopaminergical neurones. The role of these neurones in the some modulation processes is studied. Neutral network is numeraly simulated. Modelling of the harmonic action mechanisms allow to consider stochastic resonant phenomenon and understand in definite degree a role of stochastic features of cited mechanisms. Besides, a possible new mechanism of laser-information action on bio-molecular processes is in details discussed.

P040 Early apoptotic features of K562 cell death induced by 5-aminolevulinic acid based photodynamic therapy
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The K562 cell line is a commonly used model system derived from a patient with chronic myelogenous leukaemia. K562 cells are known to be resistant to apoptosis induced by various stimuli due to the lack of p53 protein and anti-apoptotic fusion of hApoH and hApoA-1. We report on the effects of 5-aminolevulinic acid based photodynamic therapy (ALA-PDT) on K562 cells. The cell response to ALA-PDT is dose-dependent both on the irradiation time and the amount of the protoporphyrin IX accumulated in the cells during their incubation with ALA. Low-dose treatment leads to a temporary arrest of the cell proliferation. Higher doses subsequently cause mitochondrial potential dissipation along with cell swelling and then rapid increase of the plasma membrane permeability for propidium iodide. Several early apoptotic marks were detected: brood-up of the mitochondrial potential we observed moderate cytochrome-c release and the exposition of 7A6 apoptosis-related mitochondrial antigen. However, the execution phase of apoptosis did not occur: neither DNA fragmentation nor cell death were observed. We conclude that the triggered apoptotic process is interrupted upstream of the caspase cascade activation and the cell death is due to necrotic processes resulting from membrane damages.
P043 Studies on the porphyrin synthesis from ALA derivatives employed in vitro and in vivo. C. G. Perottti1, C. Casas1, H. Fukuda1, G. D. Venosa1, P. Sacca1, A. Mack Robert1, A. Batlle2, 1ICIPPV, Buenos Aires, Argentina, 2National Medical Laser Centre, University College London, London, United Kingdom.

The aim of this work was to test in vitro and in vivo the efficacy of the derivatives of 5-aminolevulinic acid (ALA): Heexy-ALA (He-ALA), Undecanoyl-ALA and R, S-ALA-2-(hydroxymethyl)tetrahydropyranoyl-ALA (THP-ALA). The compounds were tested in a cell line derived from a murine mammary tumour, in explants of tumour and in vivo, after injection of the cells into mice. Porphyrin synthesis in cells from He-ALA was more efficient than from ALA. However, Undecanoyl-ALA and THP-ALA did not improve ALA performance. The different kinetics of porphyrin synthesis from the derivatives suggest different uptake mechanisms.

Ip. injection to mice of ALA derivatives, resulted in a 4-fold lower porphyrin concentrations in tumour compared to equimolar amounts of ALA administration.

In tumour explants, porphyrin synthesis from He-ALA is similar to porphyrin synthesis from ALA, and THP-ALA induces 3.3 times lower porphyrins compared to ALA. Undecanoyl-ALA induces almost basal tetrapyrrol synthesis, indicating the correlation between both in vitro models.

When ALA levels were measured in the unperfused tumour after ALA or ALA derivatives injections, these levels did not correlate with porphyrin synthesis. This, together with the in vitro data, suggests that the capillaries are playing an important role in the entry of ALA esters into the cell.

P044 Fluorescent diagnostics of head and neck cancer with alasense. E. G. Vakulovsky1, L. V. Oumova1, G. N. Voronchova1, 1Cancer Research Center AMS of Russia, Moscow, Russian Federation, 2Organic Intermediates and Dyes Institute, Moscow, Russian Federation.

FD using Alasense [5-ALA, NIOPIC](AS) as 20% cream or biodegradable polymer have been provided in 198 patients with T1-4 skin cancers: basal cell carcinoma (BCC) - 83.4%, squamous cell carcinoma (SCC) - 13.6%, melanoma (M) - 0.04%, and in 25 patients with T1-4 oral cancer in dose 20 mg per kg of body weight. FD with detecting the borders of tumor growth and intensity of accumulation were done by Spectral-fluorescent Complex. Using light sources (l = 380 - 440 nm) we've got 2-dimensional fluorescence picture of all tumors: in 53% of skin tumors it exceeded the borders of clinically detected sites for 0.2 - 3.0 cm. The intensity of fluorescence in SCC was higher than in BCC and M. In 28.3% additional fluorescence zones were found, cytological verification in all cases. Our experience show pronounced efficacy of FD for head and neck cancer. In 10 patients with BCC PDT using dye laser've been performed with complete response in 2 cases (20%) and partial response in 8 cases (80%).

P045 Protoporphyrin IX accumulation in normal skin and basal cell carcinoma after topical or oral administration of Alasense. V. V. Sekhov1, N. N. Boulgakova (Zharikova), E. V. Filonenko, D. G. Sukhin, A. B. Marmarova, Hertzen Research Oncological Institute, Moscow, Russian Federation.

The distribution and kinetics of 5-aminolevulinic acid(ALA)-induced protoporphyrin (PP) IX fluorescence in skin of patients with basal cell skin cancer were studied in the frames of clinical trials of 5-ALA-based photosensitizer (commercial name Alasense). The group consisted of 55 patients with primary, residual and recurrent tumors. Mostly, the lesions were superficial with the diameters varied from 0.5 to 3 cm. The 5-ALA was given orally (7 patients) and topically (48 patients). The following fluorescence observations of skin were performed visually and with the help of video camera under blue light excitation (D-light System, Karl Storz GmbH, Germany). The fluorescence spectra were studied in 450-800 nm spectral range under blue (442 nm) and green (532 nm) laser light excitation. The distribution of ALA-induced PPX fluorescence in different sites of body skin were studied during the first 48 hours after oral application of 5-ALA (50 mg/kg body weight) and during the first 24 hours after 6 h and 12 h topical application. The correlations between ALA-induced red fluorescence of tumor, its size and growth type were studied as well as the correlation between the tumor fluorescence and PDT response.

P046 Does surface preparation alter protoporphyrin IX fluorescence after application of 5-aminolaevulinic acid to skin cancers? S. H. Ibbotson1,2, H. Moseley1,2, L. Brancancone1,2, 1University of Dundee, Dundee, United Kingdom, 2University of Dundee, Dundee, United Kingdom.

Photodynamic therapy (PDT) using 5-aminolaevulinic acid (ALA) is increasingly used to treat skin cancers. Tumour-localised protoporphyrin IX (PpIX) fluorescence is visible after 4-6 hour ALA application. Variation exists in methodology and, although surface preparation of the lesion is commonly performed, this is not standardised. The rationale for surface preparation is to improve ALA uptake, however there are no data to indicate that this is beneficial. We used in vivo fluorescence spectroscopy using excitation at 405 nm, to determine the effect of surface preparation on PpIX fluorescence. Ten lesions of superficial basal cell carcinoma or Bowen's disease were studied. Lesions were subdivided into halves, which were randomised to either abradecutقارة or no surface preparation. Spectra were recorded with a homemade diode-array spectrometer using the output of an irradiation monochromator as the source of optical excitation and a bifurcated fibre to deliver excitation light and record fluorescence.

Blinded assessments showed no significant effect of surface preparation on absolute PpIX fluorescence. However, the ratio of PpIX to autofluorescence was significantly higher with surface preparation (p<0.01). In summary, surface preparation does not appear to alter PpIX accumulation but may influence autofluorescence. The clinical relevance of these findings needs to be examined.

P047 In vivo kinetic and photodynamic efficacy after intravesical instillation of 5-ALA. S. El Khattabi1, M. D’Hallewin2, J. Dileo2, A. Leroux2, D. Notter2, C. Vigneron3, F. Guillemmin4, 1Henri Poincare University, Nancy, France, 2Alexis Vautrin Centre, Vandoeuvre l’Est, Nancy, France.

The kinetic of PpIX accumulation was studied in vivo by fluorescence spectroscopy. Two hALA concentration (8, 16 mM) were instilled intravesically in normal and orthotopic tumor bladder model for 1 hour. Compared to auto fluorescence, normal bladders instilled with 8 and 16 mM show a maximum of fluorescence at 2 (2.75±5.21) and 4 hours (1.88±3.15) respectively. In tumor bladders the maximum of fluorescence was achieved after 2 (15.51±10.65) and 3 hours (10.15±8.96) of incubation with 8 and 16 mM respectively. All photodynamic assays were performed at 100 mW/cm2 with five light doses (16, 20, 25, 40 and 80 J/cm2). Histologically, light only caused no effects in normal and tumor bladders. At 8 mM hALA, normal bladders showed total urothelial denudation with focal (25 J/cm2) or total (40 and 80 J/cm2) wall necrosis. Tumors (8 mM) show total urothelium denudation and total normal wall necrosis without residual tumors at 25, 40 and 80 J/cm2. At 16 mM hALA, normal and tumor bladders show focal wall necrosis at all fluences. 8 mM hALA and 20 J/cm2 displayed tumors necrosis without normal wall necrosis. We conclude that the latter parameters (8 mM & 20 J/cm2) can be optimal to induce selective damage during PDT.

P048 Photodynamic Effects of 5-Aminolevulinic Acid and its Esters Derivatives on B-16 Cell Line. A. C. Tedesco1, R. F. Turchiel1, F. C. Vena1, J. Lavelle1, S. Pigaglio1, J. Blais2, 1São Paulo University-FFCLRP, Ribeirão Preto - SP, Brazil, 2Université Pierre et Marie Curie - LPBC, Paris, France.

Aminolevulinic acid (5-ALA)-induced protoporphyrin IX (PpIX) for Photodynamic Therapy (PDT) treatment has became in the last years a promising therapy for both neoplastic and non-neoplastic skin diseases. However, studies with topically applied 5-ALA have revealed that it shows a poor ability to diffuse through the skin structure because of its low lipophilicity. A possible solution to this problem is the use of 5-ALA derivatives. In the present work, 5-ALA esters derivatives h-ALA (hexylester), o-ALA (octylester) and d-ALA (decylester) were synthesized and the potential induction of PpIX production in vitro had been compared with 5-ALA. o-ALA and d-ALA was reached at the optimal concentration: 0.3, 0.075, 0.1 and 0.075 mM respectively. The results indicated that comparable PpIX fluorescence induction was achieved by using lower doses of the 5-ALA, the only exception observed was with d-ALA. The cellular viability for the derivatives after irradiation showed the same behavior detected by 5-ALA, but with much lower drug concentration. Supported by FAPESP and UOF-COPECUB.
Photodynamic therapy using delta aminolaevulinacid has been developed as a feasible method to treat superficial malignations especially in elderly people. Skin tumors, like actinic keratoses, superficial squamous cell carcinoma, basal cell carcinoma, morbus bowen are treated by topical application of 5-aminolevulinic acid (ALA) induced PDT at the Department of Otorhinolaryngology of the National Medical Center, Budapest, Hungary, 2Photoscience Laboratory, Budapest University of Technology and Economics and Chemical Research Center, Budapest, Hungary.

Photodynamic therapy (PDT) has become a focus of interest, all over the world, as an effective curative treatment for early carcinomas of head and neck and possibly as an adjuvant therapy of large tumours. This mode of diagnosis and treatment is based on the selective accumulation of a photosensitizer in the tumour tissue. Seven patients underwent 5-aminolevulinic acid (ALA) induced PDT at the Department of Otorhinolaryngology of the National Medical Center, Budapest. After topical application of ALA solution, four skin tumours (basal cell carcinoma) of the head and neck, two laryngeal papillomas and one intranasal invert papilloma were treated by PDT. While treating basal cell carcinoma with PDT is well established, the amount of the available information on its application for treating various forms of papillomas is considerably less. The applied light dose was 150 J·cm⁻² in the wavelength range 600-700 nm. All treatments proved to be effective, results of the follow-up over a 1 year period will be presented. The authors will describe their first experience of the use of this method in ENT in Hungary.

Photodynami...
P055  
Preclinical feasibility of fluorescence-guided tumor resection followed by metronomic photodynamic therapy (mPDT) using 5-aminolevulinic acid induced protoporphyrin IX: preliminary results

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Both fluorescence-guided resection (FGR) and Photodynamic Therapy (PDT) have been investigated, separately, aiming to respectively resect or destroy residual brain tumor after resection under white light. Both methods, separately, have demonstrated to positively influence survival. Therefore, we here investigated the preclinical feasibility of FGR followed by multiple prolonged PDT (mPDT) with long-term objective to further delay tumor re-growth and further improve survival.

In photodynamic treatment red blood cells are widely involved: They deliver the necessary oxygen to the tissue and may also realise or indicate the trans-illumination of target tissue. Indeed, the excited photosensitizer transmits its energy primarily to neighboring chemical species inducing typical reaction cascades that vary strongly from one organelle to the other. The initial results indicate the technical and surgical feasibility of FGR followed by mPDT with no negative influence of the procedure on survival. Further investigation is required to find optimum mPDT parameters that may lead to sufficient tumor cell death and thus improved survival.

P056  
Study of AlPcS4 distribution in carcinoma cells by spectrally resolved confocal laser scanning microscopy using in vivo co-incubation with fluorescent indicators

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Ever since mechanisms of photodynamic therapy (PDT) have been investigated, localization of the photosensitizer in cellular compartments has been a major issue. Indeed, the excited photosensitizer transmits its energy primarily to neighboring chemical species inducing typical reaction cascades that vary strongly from one organelle to the other. The initial results indicate the technical and surgical feasibility of FGR followed by mPDT with no negative influence of the procedure on survival. Further investigation is required to find optimum mPDT parameters that may lead to sufficient tumor cell death and thus improved survival.

P058  
Photochemical and chemical modification of gramicidin A in bilayer lipid membranes

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From a series of electrochemical and spectroscopic studies it is known that ion channels formed in bilayer lipid membranes by the tryptophan-rich peptide gramicidin A are inactivated by visible light in the presence of photosensitizers. Fluorescence measurements were used here to follow directly the damage to tryptophan residues suggested to be basic for the mechanism of sensitized photoactivation of gramicidin channels. Tryptophan fluorescence of gramicidin A in liposomes was suppressed after exposure to visible light in the presence of aluminum phthalocyanine, a potent photosensitizer. The photosensitized suppression of the gramicidin fluorescence appeared to be sensitive to singlet oxygen quencher, sodium azide, similarly to the photosensitized inactivation of gramicidin channels measured earlier by the conductance of planar lipid bilayers. Thus, the fluorescence data evidenced in favor of tryptophan photooxidation resulting from its interaction with singlet oxygen as the process responsible for the photodynamic inactivation of gramicidin channels. Chemical modification of tryptophan residues with N-bromosuccinimide was found to abolish both the gramicidin channel activity in planar bilayers and tryptophan fluorescence of the peptide in liposomes. The results of photochemical and chemical modification of gramicidin tandemly support the current view on the critical role of tryptophan residues in the gramicidin channel structure and function.

P059  
Membrane effects of symmetrical and asymmetrical porphyrin derivatives

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Porphyrin-type photosensitizers play an important role in photodynamic therapy used mainly for treatment of tumors. During this photosensitizer compound is given to tumor cells. Illumination of cells with light of proper wavelength induces photochemical reaction, which leads to cell death. One goal of research work nowadays is to reveal the mechanism of effect.

In the present work two newly developed tetraphenyl-porphyrin-derivatives were used, an asymmetrical and a symmetrical one. According to our previous examinations, the symmetrical proved to be more effective. Liposomes were made from dipalmitoyl-phosphatidylcholine and dioleoyl-phosphatidylcholine by sonication. They were containing 1 mol % spin labeled porphyrin.

The porphyrin derivative was added to it in 1 μM concentration. Illuminating the sample the intensity change of ESR signal was followed in the function of illumination time. The same types of samples were measured by differential scanning calorimetry (DSC).

Our results show that the intensity of ESR signal decreases during the illumination. The decrease curve shows exponential character. Asymmetrical deriv-atives caused more expressed decrease, but the differed expression was not significant in every case. According to DSC data the derivatives caused change of oppo-site direction in the phase transition temperature. After illumination this change became more expressed.

P060  
Water soluble hypericin complex bound to polyvinylpyrrolidone (PVP)

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Hypericin isolated from Hypericum perforatum, is an effective photodynamic substance which has been demonstrated by various studies. Lacking are practical forms of applications of hypericin solutions for introduction into body cavities and systemically. We developed an aqueous solution of hypericin non-covalently bound to polyvinylpyrrolidone (PVP). PVP is a poly-N-vinylamide of various grades of polymerization and forms of intermolecular crosslinks suitable for diagnostic and therapeutic applications. We use PVP as complex forming agent to prepare photosensitizer for photodynamic therapy and diagnostic purposes.

In pure water hypericin forms aggregates, which are nonsoluble and exhibit no fluorescence. Aqueous complex solutions of Hypericin-PVP show typical absorption spectrum and fluorescence emission band around 600 nm wavelength. These facts demonstrate presence of optimal chromophore solution without aggregation or microdispersive dispersion.

Molecular weights of PVP between 10k and 40 k are favored because they are able to diffuse through lipid cellular membranes. Intracellular binding of Hypericin-PVP-complexes is demonstrated by incubation of K562 cells followed by confocal laser microscopy observation. The dye targets membrane structures via endoplasmic reticulum within the cytoplasm and at the cell surface but not into the nucleus.

Conclusion: Hypericin forms liquid molecular chromophore complexes in water under presence of PVP thus allowing diagnostic and therapeutic applica-

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Cyclochrome derivatives of chlorin p6 – novel near-IR photosensitizers

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Cyclochrome derivatives of chlorin p6 (CICDs) are new photosensitizers absorbing in the near-IR region (705-746 nm). High photo-induced toxicity of the compounds has been revealed in AS49 human adenocarcinoma cells. Significant singlet oxygen quantum yields of CICDs have been measured in model membrane-like structures. Confocal spectral imaging (CSI) technique revealed the ability of CICDs to accumulate in living cells in a membrane-bound monomeric form. It has been shown that substituent structure affects rates of uptake and efflux of CICDs and their intracellular accumulation and localization, targeting them either to the Golgi apparatus or to the lipophylic vesicles. High photo-induced antimycin efficacy of CICDs has been observed in mice bearing subcutaneously inoculated murine lymphoma P388. It has been shown using CSI technique on frozen sections of tumors that CICDs accumulate both in tumors and in blood vessels. It was postulated that this antimycin efficiency may be caused by direct killing of tumor cells in combination with damage of blood supporting system.

The work was supported by grants of RFFI (01-04-49298, 02-04-04034, 02-04-06181, 02-04-06182), NATO (LST.CLG.977077), PICS R INTAS (01-0461).

P062
Rose Bengal acetate as a fluorogenic substrate for photosensitization: subcellular sites of location and damage, and induction of apoptosis
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In view of its potential use in photodynamic therapy (PDT) pyropheophorbide-a-methyl ester (PPME) is an attractive second-generation non-mitocondrial localizing photosensitizer. As demonstrated by Matozou et al., singlet oxygen ($1O_2$) produced by photoexcited PPME is involved in necrosis process of colon cancer cells. It was also demonstrated that photoexcited PPME can trigger apoptosis of these cells. By contrast to necrosis process, ROS other than $1O_2$ would be intermediates in the apoptosis process.

In this work, a quantitative determination of $1O_2$ and hydroxyl radical ($'OH$) production by PPME in ethanol, phosphate buffer and aqueous dispersion of small unilamellar DMPC vesicles has been undertaken by electron spin resonance (ESR) associated with spin trapping technique and absorption spectroscopy. 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was used to detect the presence of both $1O_2$ and $'OH$ in solution. Singlet oxygen quantum yield (>0.7) and $'OH$ yield (>1) have been measured by absorbance spectroscopy. Rose bengal which has a $Phi(1O_2)$ well known in ethanol and aqueous solution, was used as the standard for the quantification of PPME singlet oxygen production.

In phosphate buffer, both ESR and absorption measurements leads to the conclusion that $1O_2$ production is not detectable while $'OH$ production is very weak. In liposomes and ethanol, $1O_2$ and $'OH$ production increases strongly.

P064
ROS generation, mitochondrial changes and glutathione modulation in apoptosis induced by PDT with Purpurin-18 in H606 leukemia cells.
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Interest in Photodynamic Therapy (PDT) of tumour cells has greatly increased in recent years. We previously reported that PDT with Purpurin-18 (Pu18) induces apoptosis in HL60 (Di Stefano A. et al., 2001, Photochem Photobiol, 73(3): 290-296), through a not completely understood mechanism involving post-translational modification of some chaperones (Magi B. et al., 2002, From Genome to Proteome, 5th Siena Meeting, 284). To investigate the molecular mechanism of PDT with Pu-18, we used cytofluorimetric analysis (Ettore A. et al., 2003, J Invest Dermatol, in press) to determine the time-course of the generation of Reactive Oxygen Species (ROS), Mitochondrial Membrane Potential (Deltapsi) and glutathione content at different red light doses. Generation of ROS, depletion of glutathione and hyperpolarization of mitochondria were early events associated with PDT with Pu-18, followed by the loss of Deltapsi and induction of apoptosis, as documented by sub-diploid DNA content. All these events were red light dose-dependent. Pretreatment of cells with Ascorbic Acid (100 microM) and N-Acetyl-Cysteine (1 mM), 2 hours before irradiation, did not prevent the induction of apoptosis. The relevance of these findings in the context of the molecular mechanism of PDT with Pu-18 is discussed.
P067
Targeting of membrane lectins of tumoral cells by coated magnetic nanoparticles (NP). Application to photodynamic therapy (PDT)
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High affinity of tetrapyrrolic photosensitizers (TS) for tumoral cells is necessary for an effective PDT. NP were used to recognize suturexpressed sugar receptors on malignant cell membranes. The aim was to optimize design of glycoconjugated TS. NP were coated with b-galactosylated, a-mannosylated or b-glucosylated albumin to induce specific interactions. NP affinities were tested on three tumoral cell lines (HT29, Y76, B16).

Membrane affinity was evaluated after incubation with coated NP (1mM, 1h30) at 4°C to facilitate interactions with cell surface. Non specific interactions were evaluated using albumin coated NP. Labeled cells were selected by magnet or centrifugation. Quantification was achieved by measuring iron concentration. Affinity between B16, Y79 and galactosylated NP (7.9 ±0.5 106 NP/cells) was lowered (3.8 ±0.15 106 NP/cells) with mannosylated NP. No concentration. Affinity between B16, Y79 and galactosylated NP (7.9 ±0.5 106 NP/cells) was lowered (3.8 ±0.15 106 NP/cells) with mannosylated NP. No affinity was found for glucosylated NP (9 ±0.3 106 NP/cells). These findings were confirmed by inhibition (70%) of cellular internalization of glucosylated and mannosylated porphyrins (37°C) by the corresponding glycosylated albumin in B16 and Y79. Glucosylated porphyrins uptake was less affected.

As a conclusion, glycoconjugated albumin NP appear as a convenient tool to select glycosyl moieties to bind to TS for a better targeting of tumoral cells.

P068
Mechanism of Anti-cancer Activity of Photodynamic Drug Hypericin in Human Gioma Cells U-87 MG
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Hypericin (Hyp) displays light dependent antitumor activity for which several mechanisms have been proposed. In this contribution we show that Hyp possesses a weak dark activity on human malignant U-87 MG cell line which is closely related to its ability to inhibit the protein kinase C (PKC) activity.

Our experiments show that the cytotoxicity effect of Hyp-studied by cell viability does not depend on Hyp photoactivation up to the 50 J/cm2 light dose. Consequently the dark and/or ‘low light dose’ biological activity of Hyp in U-87 MG cells can be connected to an alternative mechanism, independent on excited state of Hyp. PKC inhibition was proposed as one of the possible alternative mechanisms. The activation of PKC leads to its approaching to the cell membrane. The PKC specific fluorescence probe fim-1 was used to monitor the PKC translocation, which reflects the activation of the enzyme. We conclude that Hyp acts as an antagonist to the PKC activator phorbol 12-myristate 13-acetate (PMA) with respect to PKC translocation. The binding constant (Kd=67 nM) of Hyp to PKC and the PKC-Hyp structural model show the possibility of the competitive binding of Hyp and PMA to Cys 2 regulatory domain of PKC.

P069
Cellular metabolization of tri-(meta-glucosyloxyphenyl)chlorin [TPC(m-O-GluOH)] : a mass spectrometry and chromatography study
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Cellular metabolization of tri-(meta-glucosyloxyphenyl)chlorin [TPC(m-O-GluOH)] should not affect the global in vitro photoactivity of G-TS. However in vivo characteristics could be altered by deglucosylation modifying amphiphilic properties, biodistribution, blood clearance, cellular interaction of the molecule.

P070
Oxidation of cellular proteins upon photodynamic treatment of cells with Hypocrellin A.
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Oxidation of intracellular proteins during photodynamic treatment (PDT) was studied using an intra cellular probe acetyl-tyramine-fluorescein (acTF). This probe labels cellular proteins, which become oxidized at tyrosine residues under the conditions of oxidative stress in a reaction similar to oxidative di-tyrosine formation. The labelled proteins can be separated with electrophoresis, followed by immunodetection of fluorescein-labelled bands. We found that PDT of rat fibroblasts, loaded with a photosensitizer Hypocrellin A, resulted in labelling of a set of intracellular proteins, which was clearly different from a set of proteins labelled upon the treatment of the cells with H2O2. The labelling patterns were further studied with 2D-electrophoresis, which showed that H2O2 treatment caused mostly labelling of endoplasmic reticulum (ER) proteins, whereas the PDT caused labelling of some ER proteins and several cytoplasmic proteins, including tubulins. We hypothesize that the pattern of the protein oxidation observed with Hypocrellin A reflects intracellular microlocalization of the photosensitizer (due to an extremely short radius of action of singlet oxygen and radicals generated upon PDT). In conclusion, oxidation-sensitive probe acTF is applicable for studying in vivo protein oxidation upon PDT, and may be useful for characterisation of protein targets of oxidation upon PDT with various photosensitizers.

P071
Foscan®-based PDT in relation to drug-light interval.
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Foscan® mediated PDT was performed in Colo26 mouse tumor allografts at different drug-light intervals (DLI) corresponding to a particular photosensitizer distribution in the tumor and plasma compartments. Animals were senzitized with 0.5 mg/kg Foscan® and treated subsequently at 1h, 24h and 96h post injection with a light dose of 10 J/cm2 (652 nm) administered at a fluence rate of 160 mW/cm2. Foscan® plasma concentration was maximum 1h after drug administration and decreased rapidly with time, while its concentration in tumor reached a plateau at 24h and remained constant until 96h. Tumors subjected to illumination 24h after senzitation exhibited a drastic growth delay compared to tumors irradiated 1h and 96h after Foscan injection. Therefore, plasma concentration is unlikely to be a predictive factor for PDT outcome. We show that PDT outcome is tightly bound to serum proteins, we further addressed the kinetics of the dye binding to blood cells, aiming to understand whether the cells could be the effector compartment. Flow cytometry measurements demonstrated that among DLI tested, the maximum Foscan® accumulation in white blood cells peaked at 24h post-dose. We suggest that mTHPC-PDT efficiency could be better predicted based on the photosensitizer distribution in the cells rather than in plasma or in tumor.
EMBO J protein and complete IgG of L19 antibody were conjugated to Sn-Chlorine6. Different formats (single chain Fv antibody fragment, small immunochemical conjugates) of action of immunoconjugates depends on the antibody cellular uptake of or on the chemical-physical properties of the photosensitizer. Moreover the impact of the subcellular localization on phototoxicity was investigated.

Finally the molecular mechanism underlying the mode of cell death after photoinmunotherapy with L19-SnCh6 was investigated combining confocal laser microscopy with flow cytometric analysis.

Localization and efficacy analysis of SnChlorin e6 immunoconjugates specific for extra-domain B of fibronectin

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Following the photoinmunotherapy approach, Sn-Chlorin e6 was chemically coupled to L19 recombinant antibody which selectively recognizes the extra-domain B (ED-B) of fibronectin, a tumor associated antigen. The immunoconjugate phototoxicity was tested by photoinmunotherapeutic experiments carried out on FE8 cells, which express and expose ED-B of fibronectin on the plasma membrane.

Different formats (single chain Fv antibody fragment, small immunochemical protein and complete IgG of L19 antibody were conjugated to Sn-Chlorin e6 and the subcellular distribution of these immunoconjugates was investigated by confocal laser microscopy. Very interesting was to understand if the site of action of immunoconjugates depends on the antibody cellular uptake of or on the chemical-physical properties of the photosensitizer. Moreover the impact of the subcellular localization on phototoxicity was investigated.

Finally the molecular mechanism underlying the mode of cell death after photoinmunotherapy with L19-SnCh6 was investigated combining confocal laser microscopy with flow cytometric analysis.

The PDT efficacy of 'Photosence' (0.3 mg/kg; 0.5 mg/kg) and laser irradiation (500 mV/cm2) is the most promising sensitizer. This research has been supported by the grant project of Grant Agency of the Czech Republic No. 203/02/1483 and Ministry of Education No. MSM 153100008.

The photodynamic therapy was performed by laser irradiation (670 nm, respectively 10 mW/cm2 during 10 minutes and 500 mW/cm2 during 90 seconds) following intravenous injection of 'Photosence' (0.01 mg/kg; 0.1 mg/kg; 0.3 mg/kg; 0.5 mg/kg; 0.5 mg/kg). The light beam covered the neovascular lesions and surroundings. Morphologic evaluation has not revealed any changes after isolated irradiation of different doses of 'Photosence' or isolated laser irradiation (10 mW/cm2 during 10 minutes and 500 mW/cm2 during 90 seconds). There was seen destruction of the subretinal plexus with minimal damage to overlying retina after intravenous injection of 'Photosence' (0.3 mg/kg; 0.5 mg/kg) and laser irradiation at 500 mW/cm2 (at time of 90 sec). Laser irradiation with 10 mW/cm2 light intensity (at time of 10 min) after intravenous injection of the same dose of 'Photosence' and laser irradiation at 500 mW/cm2 light intensity during 90 seconds after injection of 0.01 mg/kg 'Photosence' have not do any harm.

Conclusion: Isolated "Photosence" injection or isolated laser irradiation do not effect on retinal tissue. The ocular damage occurs only in combination of "Photosence" (0.3 mg/kg; 0.5 mg/kg) and laser irradiation (500 mW/cm2). The severity of this damage depends on "Photosence" dose.

The PDT was provided irradiating indocyanine green (ICG)-preloaded MCF-7 cells with an InfraRed laser; chemotherapy was supplied by cis-Platin. We observed that most cells necrotize, while a number of them undergoes apoptosis without changes in p53 and p21 expression. We report the ensemble of mechanisms by which the combination of chemotherapy-PDT elicit cytotoxic effects in vitro on malignant MCF-7 cells.

The PDT was performed in combination with cis-Platin. We report the effect of both individual and combined treatments (PDT, cis-Pl and PDT + cis-Pl, combined) on cell growth and viability (Trypan Blue exclusion, MTT and Colony Forming assays) and on proliferative/metabolic events as Thymidine and Methionine incorporation and basal vs insulin-mediated Deoxy-glucose uptake.

Work supported by grant from a Programma Rilevante Interesse Nazionale (2000) – MIUR - Italy.
Photodynamic Therapy

Synthesis of Halogenated Benzenophenazine Dyes for Application in Photodynamic Therapy

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Phenothiazine and phenoxazine dyes are known for their photophysical properties and some derivatives find application as laser dyes and also in the medical area, e.g. for virus inactivation of blood. Another possible field of application of these dyes is Photodynamic Therapy (PDT), a particular area of photochemistry which depends on the combination of three components, visible light, a photosensitizing drug and molecular oxygen, in order to produce biological damage of therapeutic value.

For most photosensitizers to be effective in PDT, it is essential that there is efficient intersystem crossing to the triplet state, which is then able to transfer energy to cellular triplet oxygen to produce singlet oxygen. Therefore, photosensitizers must have long-lived triplets (τ > 100 ms, Δ τ > 0.4) in order to produce singlet oxygen with good quantum yields (φτ > 0.2). Cationic dyes have received attention because of their selective uptake by mitochondria of cancer cells.

We have examined the singlet oxygen sensitizing effectiveness of these new phenothiazine and phenoxazine dyes in solution and the results from these experiments indicate that oxazine derivatives are generally ineffective at generating singlet oxygen, and although the phenothiazines examined were much better sensitizers, none of them was as efficient as methylene blue.

We are interested in developing new cationic halogenated phenoxazines as candidates for PDT. Phenoxazine dyes have been prepared by reaction of iodobenzoic acid with 5-dialkylamino-2-nitrosophenols, purified and characterized by elemental analysis and spectroscopic methods.

P080

PDT and fluorescent diagnostics with radaclorine in skin cancer patients.

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PDT and fluorescent diagnostics with radaclorine in skin cancer patients.

I. C. Moun, J. M. Martin;

Department of Quimica, Braga, Portugal.

Photodynamic therapy (PDT) and fluorescent diagnostics (FD) using radaclorine with 5-dialkylamino-2-nitrosophenols, purified and characterized by elemental analysis and spectroscopic methods.

We investigated the efficiency and the mechanism of action of a tetrathenylporphyrin derivative in photoreaction with T7 phage as surrogate of non-enveloped DNA viruses. TPF2 was able to sensitize the photoactivation of T7 phage in spite of the lack of its binding to the nucleoprotein complex. The efficiency of TPF2 sensitization was limited by the aggregation and by the photobleaching of porphyrin molecules. Addition of sodium azide or 1,3-dimethyl-2-thiourea to the reaction mixture moderated T7 inactivation, however neither of them inhibited T7 inactivation completely. This result suggests that both type I and type II reactions play a role in the virus inactivation.

Optical melting studies revealed structural changes in the protein part but not in the DNA of the photo-chemically treated nucleoprotein complex. Polymerase chain reaction also failed to demonstrate any DNA damage. CD spectra of photosensitized nucleoprotein complex indicated changes in the secondary structure both of the DNA and proteins. We suggest that damages in the protein capsid and/or loosening of protein – DNA interaction can be responsible for the photodynamic inactivation of T7 phage. The alterations in DNA secondary structure might be the results of photochemical damages in phage capsid proteins.

P082

Photosensitized inactivation of T7 phage as surrogate of non-enveloped DNA viruses

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We investigated the efficiency and the mechanism of action of a tetrathenylporphyrin derivative in photoreaction with T7 phage as surrogate of non-enveloped DNA viruses. TPF2 was able to sensitize the photoactivation of T7 phage in spite of the lack of its binding to the nucleoprotein complex. The efficiency of TPF2 sensitization was limited by the aggregation and by the photobleaching of porphyrin molecules. Addition of sodium azide or 1,3-dimethyl-2-thiourea to the reaction mixture moderated T7 inactivation, however neither of them inhibited T7 inactivation completely. This result suggests that both type I and type II reactions play a role in the virus inactivation.

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P083

Specific binding does not mean selective photochemical damage

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Cationic porphyrins which can attain a planar configuration are considered to bind non covalently to DNA by two distinct mechanisms: intercalation between base pairs or external binding in a groove. DNA was also identified as the main subcellular binding site of meso-tetra-(4N-methyl-pyridyl)porphyrin (TetraMPyP).

We investigated the binding of TetraMPyP to natural DNA and nucleoprotein complex. We selected DNA isolated from T7 bacteriophage and T7 particle as nucleoprotein (NP) complex.

Using absorption spectroscopy, conventional and time resolved fluorescent spectroscopy we identified the intercalated end externally bound porphyrin in the TetraMPyP – DNA complex. Technique used earlier gave only a global apparent binding constant irrespective of the mode of binding to natural DNA. We suggest a method based on a multi-peak fitting of absorption spectra recorded at various base pair/porphyrin ratios to quantify the different binding forms of porphyrin.

Analysing the TetraMPyP – NP complex two binding forms of porphyrin could be supposed. However, results of optical melting studies suggest that TetraMPyP specifically bind to DNA and not to protein part of NP complex.

In spite of specific DNA binding of porphyrin, thermal stability of phage protein capsid was also decreased, when T7 was irradiated with white light in the presence of TetraMPyP.

P084

Mechanism and efficiency of photodynamic inactivation of T7 phage sensitized by cationic porphyrins

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The photo-chemical treatment is a promising approach in virus inactivation and consequent disinfection of blood products. Recently cationic porphyrins were suggested as potential viral photosensitizers due to their DNA binding ability.

We investigated photosensitising efficiency and mechanism of action of free-base meso-substituted (4N-methyl-pyridyl)porphyrins holding 1, 3 or 4 positive charges and Fe- tetraMPyP. T7 phage was used as a model of non-enveloped DNA viruses. Phage inactivation was measured as the function of base pair/porphyrin ratio, porphyrin concentration or incident dose. The efficiency of porphyrins followed the next order both in their dark and photoreaction: Fe-tetraMPyP>monoMPyP>Fe-tetraMPyP. It was found that the phage inactivation cross section is higher for free then for bound tri-MPyP and tetraMPyP.

Structural changes of nucleoprotein complex and isolated T7 DNA in dark- and photo-reaction were followed by optical melting measurements. Dark binding of porphyrins slightly influenced the thermal stability of protein capsid; DNA was stabilised by the presence of tri-MPyP and tetraMPyP. In photoreaction tri-MPyP and tetraMPyP similarly destabilised T7 proteins, but the effect for DNA stability was significantly higher in the case of tetraMPyP.

Under oxygen-free conditions the efficiency of the photoeffect was smaller but still significant.
P087 Photodynamic injury of crayfish mechanoreceptor neuron and satellite glial cells
M. S. Kolosov, D. E. Bragin, A. B. Udzensky; Rostov State University, Rostov-on-Don, Russian Federation.

Potential PDT application for treatment of brain tumors including gliomas is currently studied. However, PDT effect on normal glial cells is unknown. We used a simple model system – isolated crayfish mechanoreceptor consisting of receptor neuron and satellite glial cells. Sulphonated aluminophosphalcanine Photosens was localized predominately in the glial envelope around the neuron. PDT treatment with 10^7 M Photosens inhibited and then irreversibly abolished neuronal activity for approximately 20 min. Then, in 1.7 h after PDT, the plasma membrane lost its integrity and extracellular propidium iodide staining) in a half of the neurons and glial cells just during irradiation and the dose used was 2, 4 and 6 Gy. Cytotoxicity was assayed using colony forming ability of cells.

Our results show that Indocyanine green induces cell death following phototoxification but it does not act as radiosensitizer when used with ionizing radiation.

The combined treatment of photodynamic therapy and radiotherapy produces an additive effect that does not depend on the sequence of two treatments and it is useful because it allows to reduce ionizing radiation dose to cause the same effect of RT treatment alone.

P085 On the role of some signalling pathways in photodynamic inactivation of isolated crayfish neuron
A. B. Udzensky, D. E. Bragin, M. Kolosov; Rostov State University, Rostov-on-Don, Russian Federation.

Involvement of some signalling pathways in the response of isolated nerve cell to PDT effect of sulphonated aluminium phthalocyanine Photosens has been studied. Photostimulation of an isolated crayfish stretch receptor neuron with 10^7 M Photosens gradually inhibited firing and irreversibly abolished neuronal activity. Activation of protein kinase C by phorbol ester TP, inhibition of protein phosphatases by sodium orthovanadate or calcium, or inhibition of adenylylcyclase by DLD-1330A, or inhibition of tyrosine protein kinase by genistein, or elevation of cytosolic Ca2+ concentration by ionomycin or thapsigargin shortened neuron lifetime. In contrast, inhibition of protein kinase C by staurosporine, hypericin or chelerythrine, or activation of adenylylcyclase by forskolin, or inhibition of protein phosphatases by sodium orthovanadate or calcium, or inhibition of phosphorylasekinol 3-kinase by wortmannin or LY294002 protected neuron and increased its lifetime. Therefore, protein kinase C, phosphorylasekinol 3-kinase, adenylylcyclase, tyrosine protein kinase, protein phosphatase and Ca2+ were involved in PDT-induced neuronal death and photokinetics.

The work was supported by RFBR, grants 02-04-48027, 03-040-06101 and 03-04-06102.

P086 PDT and RT: combined effects on human prostate cells in vitro
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A lot of interest is addressed to the development of new therapeutic treatments of prostate cancer that combine the radiotherapy with chemical agents and, recently, with the photodynamic therapy (PDT).

Human prostatic cells growth has been examined after photodynamic therapy, radiotherapy and combined treatment. Indocyanine green was used as photosensitizer for PDT and tested as radiosensitizer for RT. The light source used for the photostimulation experiments was a diode laser peaked at 805 nm. The cells were then irradiated for 15 minutes with a fluence rate of 108 J/cm2. Ionizing radiation was produced by a linear accelerator and the dose used was 2, 4 and 6 Gy. Cytotoxicity was assayed using colony forming ability of cells.

Our results show that Indocyanine green induces cell death following phototoxification, but it does not act as radiosensitizer when used with ionizing radiation.

The combined treatment of photodynamic therapy and radiotherapy produces an additive effect that does not depend on the sequence of two treatments and it is useful because it allows to reduce ionizing radiation dose to cause the same effect of RT treatment alone.

P088 Photodynamic effect of riboflavin on isolated crayfish stretch receptor
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Endogenous flavins photosensitize various intracellular processes. The photodynamic effect of the exogenous riboflavin on isolated crayfish mechanoreceptor neuron and satellite glial cells was studied in the present work. The preparation was incubated with 100 μM riboflavin and irradiated with a filtered blue light (400-550 nm, 0.25 W/cm2) of a tungsten lamp. Such PDT treatment damaged the plasma membrane (monitored by propidium iodide staining) in a half of the neurons and glial cells just during irradiation and the number of damaged cells increased after PDT. These cells were classified as necrotic. Bioenergetic processes (monitored by the succinate dehydrogenase activity) were not significantly impaired at the moment of abolition of neuron activity, but were significantly inhibited 4 h after PDT. Apoptotic fragmentation of the neuron nuclei was not observed, though the number of apoptotic glial cells significantly increased after PDT treatment. Therefore, electrophysiological changes in the neuronal activity were the first hallmark of PDT-induced cellular damage.

The work was supported by RFBR, grants 02-04-48027, 03-040-06101 and 03-04-06102.

P089 Azaphorpecenes: new photosensitizing macrocycles with improved near-IR absorption

The need for new PDT photosensitisers with intense absorption in the range 700-800 nm has estimulated the search of new compounds that satisfy this objective. Using computational chemistry techniques our group identified the 2,7,12,17-tetra- (p-butylyphenyl)-3,6,13,16-tetraazaporphycene(1) as an interesting candidate, predicting a bathochromic shift of 80 nm relative to 2,7,12,17-tetraphenylporphycene(2). This compound was synthesized successfully(2) and the bathochromic shift was confirmed. Indeed, this compound shows distinct absorption bands in the red, where the furthest is at 760nm with an ε = 2.04·10^4 M^1·cm^-1. The fluorescence maximum appears at 777 nm and it is reached with a quantum yield of 0.03. The singlet state lifetime is 0.5 s. The formation of a long-lived triplet state (τt = 40 μs in argon-saturated toluene) allows the formation of the reactive singlet oxygen albeit with a low quantum yield (φt=0.013). Work is currently in progress to improve these properties which may prove a valuable addition to the field of PDT photosensitisers.


P090 Photophysical and photochemical characterization of amino acid and peptide derivatives of N,N′-bis(2-phosphono ethyl)-1,4,5,8-naphthalenediimide synthesized on silica particles
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In the search for new phototoxic supramolecules that could be of use in photodynamic therapy we synthesized on silica particles the amino acid (Tryptophan, Thr) and peptide derivatives (Ac-Tp-Ala-Ala-OH) of N,N′-bis(2-phosphonoethyl)-1,4,5,8-naphthalenodiimide (DPN).

The DPN molecules were bound to the particles using zirconium/phosphate chemistry and the aminoacids were bound to the DPN-silica particles by using F-moc solid state synthesis. Spectroscopic studies and molecular modeling showed that the Trp residue in the Ac-trp-alta-alta-DPN-silica derivative tends to interact with the alamine residues. Therefore, the triple in this derivative is located in more apolar environment than the Trp residue in the Ac-Trp-DPN-silica derivative. Trp and DPN species form a noncovalent photoactive complex in aqueous solution.

The silica derivatives of trp (DPPN) complexation is observed. Laser flash photolysis studies of these particles showed that DPN is photosensitizing the formation of tryptophan cation radical and tryptophan neutral radical, by forming DPN anion radical. The molecular organization of these suprastructures in the silica particles stabilize the tryptophan cation radical avoiding the fast charge recombination with the DPN anion radical. Our studies show that the rate of triplet and radical formation by the sensitizer can be modulated by the distance from the Trp residue.
Photodynamic efficacy of hypericin in skin keratinocytes using different clinically available light sources emitting in the red and visible range. The lower yield of electron transfer observed for dimers in AOT micelles and remain within the cell up to 6 h after removal of hypericin from the culture medium containing the perinuclear fraction as well as the melanosomal fractions of, both, PDT-treated (melanin-high) and untreated (melanin-low) cells. These data indicate that PDT-induced melanin production was not prevented by the addition of cycloheximide or actinomycin D or of the tyrosinase inhibitor L-depenyl. In vitro, these cells produce only little melanin. However, after PDT we found a dramatic elevation in intracellular melanin. Melanin production increased with, both, the concentration of the sensitizing agent and the light dose, and was found to continue for several hours after cell death. PDT-induced melanin synthesis was not prevented by the addition of cycloheximide or actinomycin D prior to irradiation, indicating that de-novo protein synthesis and the transcriptional activity are not required for this effect. We also analyzed tyrosinase activity, a key enzyme in melanin biosynthesis, in PDT-treated B16 cells. Tyrosinase activity was found in PDT-treated as well as untreated cells. Cell fractionation experiments showed that tyrosinase was present in the cytosol as well as the melanosomal fractions of, both, PDT-treated (melanin-high) as well as untreated (melanin-low) cells. These data indicate that PDT-induced production of melanin is not controlled at the transcriptional or translational level and that tyrosinase is not an essential regulator in this process.

Photodynamic efficacy of hypericin in skin keratinocytes using different clinically light sources.

Hypericin, a component of St. John’s Wort, is a potent natural photosensitiser, and may have potential in photodynamic therapy (PDT), which uses a photosensitiser, oxygen and visible light to kill tumour cells. Hypericin has a broad absorption spectrum encompassing the UV and visible portions of the electromagnetic spectrum. We have compared the efficacy of hypericin-PDT using different clinically available light sources emitting in the red and yellow-green portion of the visible light band (PDT1200 & PDT1200SOA, Waldmann). Our results show that hypericin accumulation in the perinuclear region of HaCaT cells is apparent after 30 minutes incubation with the drug, and remains within the cell up to 6 h after removal of hypericin from the culture medium. We found no significant differences between the two light sources when hypericin-PDT induced phototoxicity, photogenotoxicity and generation of apoptotic cells was examined. DNA damage did not correlate with either the total percentage of cell viability or the fraction of apoptotic nuclei. This observation supports the idea that DNA is a secondary effect of PDT, and not directly involved in photodynamic killing. These results show that hypericin-PDT is extremely effective, if not more so, than porphyrin-based photosensitisers at killing non melanoma skin cancer cells.

Influence of Negatively Charged Interfaces on the Ground and Excited State Properties of Methylene Blue

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Properties of the ground and excited states of methylene blue (MB) were studied in negatively charged vesicles, normal and reverse micelles and sodium chloride solutions. All these systems induce dimer formation as attested by the appearance of the dimer band in the absorption spectra (λobs ~ 600 nm). Differences in the fluorescence intensity as a function of dimer/monomer ratio as well as in the resonance light scattering spectra indicates that distinct types of dimers are induced in SDS micelles and AOT reversed micelles. The properties of the photo-induced transient species of MB in these systems were studied by time-resolved NIR emission spectra, by laser flash photolysis (transient spectra, yield and decay rate of triplets) and by thermal lensing (amount of heat deposited in the medium). The competition between electron transfer (Dye-Dye*) and energy transfer (DyeO2-Dye) reactions was accessed as a function of the dimer/monomer ratio. The lower yield of triplet emission observed for dimers in AOT micelles and intact vesicles compared with SDS micelles and reverse vesicles at similar dimer/monomer ratios is related with the different types of aggregates induced by each interface.

Photodynamic Treatment Induces Melanin Synthesis in B16F1 Melanoma Cells

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The success of photodynamic therapy (PDT) of melanocytic tumors is severely limited by insufficient penetration of light into deeper tissue layers. In this study, we analyzed the effect of PDT on the melalin production of the melanoma cell line B16F1. In vitro, these cells produce only little melanin. However, after PDT we found a dramatic elevation in intracellular melanin. Melanin production increased with, both, the concentration of the sensitizing agent and the light dose, and was found to continue for several hours after cell death. PDT-induced melanin synthesis was not prevented by the addition of cycloheximide or actinomycin D prior to irradiation, indicating that de-novo protein synthesis and the transcriptional activity are not required for this effect. We also analyzed tyrosinase activity, a key enzyme in melanin biosynthesis, in PDT-treated B16 cells. Tyrosinase activity was found in PDT-treated as well as untreated cells. Cell fractionation experiments showed that tyrosinase was present in the cytosol as well as the melanosomal fractions of, both, PDT-treated (melanin-high) as well as untreated (melanin-low) cells. These data indicate that PDT-induced production of melanin is not controlled at the transcriptional or translational level and that tyrosinase is not an essential regulator in this process.

Photodynamic Therapy: A promising approach in development of new photodynamic therapy protocols

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In most photosensitization protocols using merocyanine-540 (MC540) this low-permeant dye causes extensive photodamage to the plasma membrane (PM) resulting in cell death preliminary via necrotic pathway. In our studies, we used prolonged (up to 3 h) incubation of cells in dye-containing medium (0.01 mM MC540) with subsequent washings in serum containing buffer to address the MC540-induced photodamage mainly to the intracellular sites rather than PM. High (> 50%) percentage of apoptotic death was achieved in rat thymocytes and bone marrow cells and human K562 erythroblasts. The staining with rhodamine-123 and neutral red demonstrated the decrease in mitochondrial potential and the increase in lysosomal pH in illuminated (λmax = 690 nm-collimated light induced a 3-fold decrease of tumor growth rate. However, a total regression was not observed in our model. To sum up, these data suggest that photodynamic therapy with Visudyne may have a role in the treatment of pigmented melanomas and need more studies.

Photofrin® uptake in human oesophageal carcinoma and healthy oesophageal tissue: is there a difference?

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The aim of this study was to evaluate uptake of Photofrin® in oesophageal carcinoma and normal oesophagus. Biopsies from carcinoma and normal oesophagus were taken, from patients undergoing Photofrin® induced photodynamic therapy (PDT). These were weighed, added to 1 ml 1M sodium acetate-acetic acid (4:1 v/v) and centrifuged. The organic phase was decanted, mixed with 4ml 1M hydrochloric acid and centrifuged. The lower aqueous phase containing the porphyrins was retained and placed in boiling water for 30 minutes. This hydrolysed ester and ether oligomeric components into the monomeric form containing the porphyrin. The final concentration of the isolated porphyrin was measured using photometry. The absorption spectra were measured at 405 nm and the maximum fluorescence was measured at 632 nm. Fluorescence of a standard Photofrin® solution and blank were also measured.

The upper normal : carcinoma:normal ratio were calculated. The mean porphyrin concentration was 137.870 ng/mg (SEM ± 109.508) in tumour and 123.607 ng/mg (SEM ± 56.099) in normal oesophagus. The mean tumour:normal porphyrin ratio was 1.006 (SEM ± 0.295). In this study uptake of Photofrin® is similar in neoplastic and healthy tissue, with no preferential uptake observed in cancer. Despite this, effective PDT of oesophageal carcinoma can be achieved by targeting the light.
P097
Photofrin induced photodynamic therapy a useful tool in the palliation of oesophageal cancer.
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Oesophageal cancer is increasing rapidly in incidence. The main symptom is dysphagia. In patients unsuitable for surgery, endoscopic palliation is necessary. Photofrin-induced photodynamic therapy (PDT) is a promising treat- ment. The aim of this study was to investigate the effect of PDT on dysphagia, global quality of life and patient survival. Eleven patients were treated with 2mg/kg intra-venous Photofrin followed at 48 hours by the administra- tion of 635nm light. Before treatment patients completed the European Organisation for Research and Treatment of Cancer quality of life core ques- tionnaire and its oesophageal module (EORTC QLQ-C30 / OES 24). This was again completed 1 and 4 weeks after treatment. Time to treatment failure and patient survival were recorded. The median (range) pre-operative, 1 week and 4 weeks dysphagia scores were 44 (11-100), 55 (0-77) and 33 (11-66) respectively. The respective global quality of life scores were 58 (25-66), 50 (31-66) and 66 (31-83). The median time to further intervention was 62 days (range 21-98 days). Seven patients are alive after a median of 81 days (range 40-151). In those who died, the median survival was 84 days (range 77-168). This study confirms the efficacy of Photofrin-induced PDT in the palliation of oesophageal cancer.

P098
The effect of complement activation during photodynamic light delivery in the treatment of solid tumors
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Damage inflicted by photodynamic therapy (PDT) results in the release of a barrage of pro-inflammatory signals with the potential to engage the precurs- orous action of the complement (C) system against a tumor. This non-cellular arm of immunity is a key mediator of host response and wound healing and may therefore be a key regulator of the host response to a PDT-treated tumor. Comparing the PDT response of murine tumors in mice deficient in C protein C3 production with their wild-type counterpart C57BL/6, we observed that the cure rate by photofrin-PDT improved in C3 knockouts, whereas, the reverse effect was demonstrated by BPD-PDT. A sharp drop in tumor oxygen levels immediately following photofrin-PDT was observed in C57BL/6 but not in C3 knockout mice. Following BPD-PDT, however, tumor oxygen levels sig- nificantly decreased in both mouse models. These observations suggest that C-mediated vascular damage in tumors treated by photofrin-PDT, limits oxy- gen delivery during treatment thus abrogating the photodynamic process. On the other hand, direct vascular damage mediated by BPD-PDT renders C- mediated endothelial cell death irrelevant toward overall tumor cures. These studies could aid to decipher the mechanism(s) that impede tumor oxygena- tion during PDT mediated by photosensitizers that share Photofrin’s mode of action.

P099
Temperature Dependence of Collagen Fluorescence
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Dermal collagens have several fluorescent moieties in the UV and visible spectral regions. Preliminary results suggest that these fluorophores may serve as probes of collagen (supra)molecular structure. We studied the tem- perature dependence of two purified type I acid-soluble collagen prepara- tions: (1) commercial citrate soluble calf - skin collagen (Elastin Products) and (2) acid - extracted Skh -1 hairless mouse collagen. The fluorescence properties of these samples (0.5 mM in 0.5 M HOAc) were quantitatively analyzed at temperatures from 4° to 65°C for excitation/emission wavelengths 270/305 nm (tyrosine), 270/360 nm (tyrosine excimer?), 325/400 nm, and 370/450 nm (age - related glycosylated product?). L - tyrosine (1 x 10^-4 M in 0.5 M HOAc) was actuated as reference. Reciprocal plots of 1/λ exc vs 1/T (°K) afforded straight lines at T < Tm, whereas they were generally nonlinear for T > Tm. The degree of nonlinearity varied with sample and chosen probe wavelengths. These results indicate that the fluorophores are at different loci on the collagen backbone and are thus differentially affected by helix/coil transitions. Additional studies could possibly provide insight into collagen 3D structure in vivo. Supported by NIH/MBRS Grant # GM08248 and ERMCI Grant # 03034.

P102
Progress toward defining the structure of melanin
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Natural melanins are complex biosystems containing at least three con- stituents: metal ions, proteins, and organic molecules. Of the melanins pres- ent in the human body, only hair melanin provides sufficient quantities of material to allow for physical and chemical characterization. To investigate the assembly of the organic constituent, electrospray ionization mass spec- trometry methods have been employed on human hair eumelanin isolated by enzymatic extraction. Separation of the complex mixture by liquid chro- matography is necessary to obtain interpretable mass spectra. Further struc- tural information about these species can be elucidated through fragmenta- tion patterns afforded by ion trap detection.

P103
Secretion of interleukin-1α by human skin equivalents treated topically with chlorpromazin and exposed to UVA radiation
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Human skin equivalents exposed to 7.5 joules/m² of UVA remained 100% viable. When treated topically with chlorpromazin (0 to 3mM) for 18 hours (37°C, 95 % ambient air, 5 % CO2) and transferred to fresh medium for 24 hours, viability dropped from 100% at 0 mM to 90% at 3 mM. IL-1α secretion is considered to be the main epidermal eicosanoid, and is assumed to have both pathophysiologic effects in inflammatory skin diseases as well as a physiological role in cutaneous biology. Therefore, we will present our model that UV-light effect on 12-HEET cell surface receptors in 2 different keratinocyte sources: in normal chronically sun exposed skin keratinocytes and in keratinocytes with no sun exposure history obtained from vaginal mucosa. Therefore, in the pres- ent work we studied the effects of single and repeated irradiations with selected UV-B light of 311 nm on the 12-(S)-HEET receptors in keratinocytes.

P100
Modifications of in vitro skin penetration in response to solar irradiation: evaluation on flow-through diffusion cells
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The effect of solar irradiation on ex vivo dermatomic hairless rat skin sam- ples maintained in culture on flow-through diffusion cells for 24 hours was demonstrated by MITT assay and histologic observation. Trans-epidermal water loss (TEWL) measurements and the kinetic analysis of the permeation of both tritiated water and 14C caffeine through the skin were performed after full-spectrum solar exposure. After UV exposure of less than 210 mJ/cm², skin integrity and the permeation of both water and caffeine did not change significantly. In contrast, after a 400 mJ/cm² irradiation, the epidermis appeared more contracted, and there was an increase in TEWL of 55 %. After 6 hours, there was skin permeation of 220 % of tritiated water. After 12 hours, the flux of 14C caffeine increased rap- idly to 338 %.

P101
The UV-light of 311 nm effect on 12(S)-hydroxyeicosatetraenoic acid receptors in normal skin keratinocytes as compared with normal mucosal cells
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UV-light induces different changes in skin. On the cell surface, receptors could be also affected during treatment with artificial UV sources or during skin irradiation for cosmetic reasons. Because 12-hydroxyeicosatetraenoic acid (12-HETE) is considered to be the main epidermal eicosanoid, and is assumed to have both pathophysiologic effects in inflammatory skin diseases as well as a physiological role in cutaneous biology, we decided to study the UV-light effect on 12-HEET cell surface receptors in 2 different keratinocyte sources: in normal chronically sun exposed skin keratinocytes and in keratinocytes with no sun exposure history obtained from vaginal mucosa. Therefore, in the pres- ent work we studied the effects of single and repeated irradiations with selected UV-B light of 311 nm on the 12-(S)-HEET receptors in keratinocytes. No significant difference between non-irradiated keratinocyte receptor num- bers (Bmax) in both cell types was seen. UV-irradiation in vitro (50-150 J/m²) induced a down-regulation of 12(S)-HEET receptors in a dose-dependent man- ner. The receptor affinity remained unchanged in both cell types. These results may hypothesize a presence of a hardening factor which may partial- ly prevent the receptor down-regulation in chronically sun exposed ker- atinocytes. Further experiments supporting this hypothesis are in progress in our laboratory.

P104
Temperature dependence of collagen fluorescence
I.M. Menter
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Dermal collagens have several fluorescent moieties in the UV and visible spectral regions. Preliminary results suggest that these fluorophores may serve as probes of collagen (supra)molecular structure. We studied the tem- perature dependence of two purified type I acid - soluble collagen prepara- tions: (1) commercial citrate soluble calf - skin collagen (Elastin Products) and (2) acid - extracted Skh -1 hairless mouse collagen. The fluorescence properties of these samples (0.5 mM in 0.5 M HOAc) were quantitatively analyzed at temperatures from 4° - 65°C for excitation/emission wavelengths 270/305 nm (tyrosine), 270/360 nm (tyrosine excimer?), 325/400 nm, and 370/450 nm (age - related glycosylated product?). L - tyrosine (1 x 10^-4 M in 0.5 M HOAc) was actuated as reference. Reciprocal plots of 1/λ exc vs 1/T (°K) afforded straight lines at T < Tm, whereas they were generally nonlinear for T > Tm. The degree of nonlinearity varied with sample and chosen probe wavelengths. These results indicate that the fluorophores are at different loci on the collagen backbone and are thus differentially affected by helix/coil transitions. Additional studies could possibly provide insight into collagen 3D structure in vivo. Supported by NIH/MBRS Grant # GM08248 and ERMCI Grant # 03034.
Photodynamic therapy (PDT) uses light to activate a photosensitizer, which then generates reactive oxygen species (ROS) that can damage cancerous cells. The goal is to selectively deliver light to the tumor site and use the photosensitizer to convert light into ROS, thereby destroying cancer cells without harming healthy tissue. This approach is advantageous in treating superficial or minimally invasive tumors and can be combined with surgery or radiotherapy.

Key aspects of PDT include:
1. **Sensitizer Selection**: Choosing a photosensitizer with high light absorption and ROS production efficiency.
2. **In Vivo and In Vitro Analysis**: Studying the distribution, pharmacokinetics, and phototoxicity of the sensitizer to optimize treatment efficiency.
3. **Dosage and Time**: Determining the optimal dosage and time delay between injection and irradiation to achieve maximum therapeutic effects.
4. **Patient Monitoring**: Continuous monitoring of patients to assess safety and efficacy.

PDT is used in various medical fields, including dermatology, ophthalmology, and oncology. It offers the potential for minimally invasive treatment with less scarring compared to traditional surgical methods.

In conclusion, photodynamic therapy is an evolving field that continues to demonstrate promise in cancer treatment. Ongoing research is necessary to improve the efficacy and minimize side effects, making PDT a valuable tool in modern oncology.

References:

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**P105**

**Pyridoxal-Phosphate-Sensitized Photoactivation of Tryptophanase**

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The high photostability of the active site-bound pyridoxal phosphate (PLP) in tryptophanase (Tnase) revealed marked changes in the fluorescence and absorption spectra upon irradiation of enzyme solutions at pH 8.0 and in the presence of potassium ions, i.e., when the 337-nm species (active form of Tnase) predominates. A correlation between enzyme inactivation and PLP photomodification was observed.

**P106**

**Photostability and Phototoxicity of Hydrocortisone acetate**

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Hydrocortisone acetate is a corticosteroid used systemically for the treatment of rheumatoid arthritis, endocrine disorders and allergic diseases, and topically for dermatoses as an antiinflammatory and antiallergic agent.

**P107**

**Photostabilities and in vitro phototoxicity studies of several compounds used as sunscreen agents.**


The effect of commonly used infusion media on the photostability of epinephrine is a topic that has so far received modest attention. The photostability effects on epinephrine of the medium used are discussed. Media effects on the photostability of epinephrine are commonly prepared in combination with sulfites (metabisulfite, bisulfite, sulfite). Sulfites are added to protect epinephrine from oxidation. It has, however, been shown that sulfites may induce the degradation of epinephrine when exposed to light. The photodestabilisation of epinephrine is caused by bisulfite reacting with degradation products of epinephrine forming a photosensitizer (adenochrome sulfonate). The photosensitizer has been shown to produce singlet oxygen.

**P108**

**Photophysical properties of the antibiotic tetracycline**

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Tetracyclines (TC) are a class of phototoxic antibiotics whose bioactivity depends on their complexation ability with metal ions, such as magnesium and calcium ions, and with biomacromolecules, in particular RNA. TC is also known to adopt different steric conformations depending on the nature of the solvent and complexing agent. The present contribution aims at a comprehensive characterization of the photophysics of TC in different media, as well as of its complexes with magnesium ions, calcium ions, and/or RNA. The spectroscopic and excited state properties of TC have been analyzed by means of absorption, steady-state and time-resolved emission, transient absorption, and circular dichroism spectroscopies.
Photodynamic Action of some Sensitizers by Photooxidation of Luminol

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The photosensitization of luminol, a synthetic guaiacol-based phenoxazinone, is well-known. Its photooxidation by red or near-infrared laser light is due to the production of powerful one-electron oxidants (singlet oxygen) which react with luminol producing an oxidation product, luminol oxide (LO), which is a fluorescent and anionic species. The singlet oxygen production depends on the properties of the used sensitizer. The LO formation is a useful tool for detecting singlet oxygen by means of fluorescence spectroscopy.

A new simple experimental method which can be applied to compare new photosensitizers on the basis of their production of reactive oxygen species has been developed. A high-performance liquid chromatography (HPLC) assay allows monitoring of several substances (sensitizer, reactant, oxidized end product) simultaneously on a single chromatogram. Photoreactions were monitored throughout their course by the HPLC assay to survey the sensitizing properties of Betamethasone and its stereoselective photobinding to protein.

Therefore, both the loss of therapeutic activity and toxicity may be induced when Betamethasone is exposed to light. Bethametasone also shows photosensitizing properties; it induces photohemolysis under UVB (95 %, 1,5 J/cm²) and UVA light (55 %, 30 J/cm²). The isolated photoproducts also photohemolyze the RBC in the order of potency: 1c > 1d, thus proving to contribute to the overall photosensitizing properties of Betamethasone. The photooxidation of the drug was also studied in different pharmaceutical formulations. The presence of additives (sodium metabisulphite, phenol) have some photoprotective effect but they do not completely preserve the drug from photolysis.

Photoallergy and phototoxicity are well-known adverse effects induced by non steroidal anti inflammatory drugs. Photoallergy and phototoxicity are well-known adverse effects induced by non steroidal anti inflammatory drugs. Carprofen has been found to be one of the most phototoxic drugs. In this aim, carprofen (R) and (S) pure stereoisomers were irradiated in solution of human serum albumin. After irradiation and gel filtration chromatography of the photomixture, the eluting protein fraction was studied by means of fluorescence spectroscopy. (R)-Carprofen sample gave rise to a structureless spectrum while (S)-Carprofen showed a more intense and well-defined spectrum. Irradiations of carprofen were run in the presence of the amino acids in order to determine which ones could be involved in the photobinding. Under these conditions, only cysteine led to a well-defined spectrum which was assigned by HPLC assay to the formation of the dechlorinated photoproduct of carprofen.

Moreover, flash photolysis experiments were run to study a possible stereoselectivity in the quenching of carprofen by human serum albumin or amino acids.

Therefore, both the loss of therapeutic activity and toxicity may be induced when Betamethasone is exposed to light.
P116 DNA controls the photosensitization of bound stilbazolium ligands
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Photosensitization is a key process exploited for the photostimulation of the function of biomaterials that enables their application in optoelectronic devices. The well-known dramatic effect of the microenvironment on the mechanism and quantum yield of isomerization suggests that DNA duplex can modulate photosensitization of properly designed ligands. We have already reported the DNA-suppressed trans→cis photosensitization of dis-
tilbazolium ligands [1].

Here, we report on the DNA-affecteda photosensitization of arylstilbazolium ligands of general formula Ar=CH=CH–Pys (where Py is N-methylpyridinium moiety, Ar is 2-naphthyl (1), 9-anthryl (2) and 9-phenanthryl (3)). Samples of dye (20 microM) in the absence and the presence of calf thymus DNA (5-500 microM) were irradiated at selected wavelengths and analyzed with a HPLC system after the DNA matrix separation. Particular isomers were identified on the basis of peak absorption spectra recorded with a diode array detector. The presence of DNA significantly affected both the photostationary state and the quantum yield of isomerization. Depending of the aryl substituent, a variety of effects were observed, including the enhancement or the suppression of isomerization as well as the formation of new photoproducts.


P117 Photoprocesses in TPPS, aqueous solutions: the role of serum albumin and pH.
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The sensitizer meso-tetra-4-sulfonatophenyl)porphine (TPPS4) in acidic aque-
ous solutions forms self-organized aggregated structures (J-aggregates). Formation and photostability of J-aggregates was investigated at pH 1 and pH 4 in the presence of different concentrations of bovine serum albumin (BSA). The size of J-aggregates in the mixed solution depended on the medium pH and the molar ratio of TPPS4 and BSA. The Soret band of TPPS4, ionic form and that of J-aggregates in the presence of BSA underwent a shift to the blue spectral region. The complex effect of BSA on the formation of J-aggregates was observed. Small concentrations of BSA boosted the process, which was reflected by the appearance of new absorption bands around 485 and 705 nm. High concentrations, however, led to its suppression or even prevented the aggregation due to the contrary interaction of TPPS4 with BSA. On the other hand, the presence of BSA didn’t disrupt the preformed J-aggregates. The phototransformation of TPPS4 in aqueous solutions was found to be strongly dependent on both medium pH and presence of BSA.

P118 Role of oxidative stress in apoptosis induced by UVA activated 8-MOP, TMA and CPZ in Jurkat cells
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Photopheresis (ECP) involves extracorporeal exposure of isolated peripheral blood leukocytes to UVA irradiation in the presence of 8-methoxypsoralen (8-MOP) followed by their reinfusion to the patient. The underlying mechanism of ECP is not well understood. In this study, we investigated the efficiency of 8-MOP, chlorpromazine (CPZ) and 4,4′-trimethylgluceline (TMA) to induce oxidative stress in Jurkat cells under the conditions similar to those that leading to cell apoptosis by TBA and hydroethidine assays. The ability of the 8-
MOP, TMA and CPZ to photogenerate singlet oxygen and oxidized cholesterol to its characteristic hydroperoxides (CHOx) in liposomal system and selected organic solvents were analyzed by time resolved 102 phosphorescence, HPLC-EC (Hg) and EPR oximetry. Our data showed that while photoactivation of all three photosensitizers induced cell apoptosis, only CPZ, under similar experimental condition led to an accumulation of MDA and oxidation of hydroethidine. We confirmed that in several organic solvents CPZ generated 102 CHOx, whereas in and in aqueous suspension of liposomes only turo-
coumarins, mainly TMA, induced measurable weak photo-oxidation of cholesterol. The data may suggest that apoptosis of the cells photoinduced by 8-MOP, TMA and CPZ and their efficiency to generate reactive species may not be related.

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In this study, we seek to understand if esterification of PpIX to PpIX dimethy-
lester (PME) would improve potential of PpIX as an exogenous pho-
sotosensitizer. NPC/NCI-2, undifferentiated human nasopharyngeal carcinoma cells, were incubated with individual photosensitizer, at different time points. Subsequently, acid/alcohol solvent was used to extract photosensitizers from cells, plasma and xenograft tissue, and quantified spectrofluorimetrically. Cellular phototoxity observed with crystal violet assay and electron microscopy, revealed that PME is a more potent photosensitizer than PpIX, at early and late time points. EM and drug localization studies shows PME tar-
geting both mitochondria and lysosome at 3h and mitochondria only at 17h. PME was also found to have a 35-47% higher uptake in vitro. In vivo, PME was found to be cleared rapidly from serum and retained longer in the tumor compared to PpIX, which had the same rate of clearance in both tumor and serum. However, PME did not show a differential uptake in the tumor com-
pared with PpIX in terms of PpIX fluorescence in this study. Hence further studies could be done to determine PME clearance, that would improve normal-tumor ratio, even though esterification of PpIX did not show a higher uptake into tumor as seen with ALA esters.

P120 Mechanism of retinyl palmitate photodecomposition
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Retinyl palmitate (RP), an ester form of vitamin A (retinol), is thermally more stable than retinol. RP is an ingredient of more than 660 cosmetic products on the U.S. market. RP photodecomposed quickly under UVA and UVB irradiation; the effect was slower under visible light. Irradiation of RP in ethanol with UVA light followed by HPLC separation and spectral analysis resulted in the identi-
fication of anhydroretinol (AR), 5,6-epoxy-RP, 4-keto-RP, two hydroxy-
ethoxy-RP isomers and palmitic acid. All these products decomposed pho-
ytolically into multiple products that did not absorb UV or visible light. AR was formed as a mixture of all-trans-RP (predominant), 6Z-cis-RP, 8Z-cis-RP, and 12Z-cis-RP. 5,6-Epoxyp-RP, 4-keto-RP, and the two hydroxy-ethoxy-RP prod-
ucts were also formed from the chemical reaction of RP with the alkyloxy-
propanal radical generated from the free radical initiator 2,2′-azo(2,4-dimethyl-
valeronitrile). This suggests photodecomposition occurred through light-
meditated free radical initiated chain reaction. Formation of all-trans-AR and palmitic acid, and the isomerization of all-trans-AR into the three cis-AR iso-
mers proceeded through an ionic photodecomposition mechanism. RP and its photodecomposition products were not photomutagenic in S. typhimurium TA100; however, both RP and 5,6-epoxy-RP were found to cause DNA single strand cleavage, while the other photodecomposition products were less or not active.

P121 Photosensitization of activated and resting human T-Lymphocytes by chlorin e6 and chlorin e6 dimethylester
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The purpose of the study was to compare the susceptibility of resting and activated T lymphocytes to photosensitization by porphyrin photosensitizers chlorin e6 and chlorin e6 dimethyl ester. First approach proposed the compari-
on of sensitizers uptake and efflux by resting and PHA-activated T lympho-
cytes. In the second one the chlorines accumulation in activated (HLADR+) and resting (HLADR-) T cells from blood of leukemia patients after bone mar-
row transplantation was compared. Sensitizers uptake studies were per-
formed using flow cytometry and spectrofluorimetry. According to obtained results chlorins uptake by PHA-activated cells was significantly higher than by resting cells. The level of chlorin e6 in activated cells was activated cells was significantly higher than by resting cells. The level of chlorin e6 in activated cells was significantly higher than by resting cells. The level of chlorin e6 in activated cells was significantly higher than by resting cells.
to previous procedures. Moreover, the detection procedure is further refined low as 100
a new and powerful non-invasive tool in form of UVA laser induced ultra-
F. M. Musumeci1, 2 G. Privitera1, 2, P.124
UVA laser photoinduced luminescence as non-invasive tool in mammalian
cells research

To study the dependence of quantum yield of the DL on the intensity and
dose of illumination, the model has been generalised to describe also the
process of illumination with account of the luminescence processes during
the time of illumination.

As a result the DL quantum yield is a non-linear function of the intensity and
dose of illumination. At relatively small intensities of the illuminating light,
the DL quantum yield increases with dose increasing, and reaches monoto-
nously some saturation at long enough time of illumination. The situation is
also to minimize the number of false-positive results and to reduce the num-
ber of biopsies which are commonly used in search for occult neoplastic
lesions. The combined approach allows to detect occult intraepithelial lesions of larynx and bronchus. The results shown that combination of endoscopic
fluorescence imaging (autofluorescence and S-ALA induced PPIX fluorescence)
with in situ spectral measurements really improves the ability to local-
ize precancerous and early cancerous lesions. The designed approach allows
to also to minimize the number of false-positive results and to reduce the num-
ber of biopsies which are commonly used in search for occult neoplastic
lesions under conventional WLB. The fluoroscopic technique has the poten-
tial to detect the occult foci of malignancy located in sinonasal lumen of larynx or bronchus.

P125
Laser metabolic conditions during transplantation: study by means of auto-
fluorescence spectroscopy and conventional biochemical techniques
A. C. Cocc, 1 S. Faroni, 1 R. Bertone, 1 B. Bertone, 1, M. Vaietti, 2 A. Ferrigno, 3 O. Neri, 1 G. Bottiroli1

Preservation-refuiperfusion phases can result in organ metabolic alterations
that contribute to graft failure after liver transplantation. The application of
autofluorescence analysis to monitor metabolism of liver during ischemic
storage were investigated. Autofluorescence spectral properties of rat livers
were characterized during transplantation under 366 nm excitation, by
means of a PMA 11-Hamamatsu with single-fiber-optic probe. Explanted livers
kept in standard storage conditions ensuring good preservation (University of Wisconsin-solution, 0°C,12h), or in conditions affecting the morphofunctional properties of the tissue (poor solution, longer ischemia)
were considered. After cold ischaemia livers were perfused with 37°C, O2
Kreb's Henseleit-medium. Autofluorescence signal increased during cold ischemia up to 180%, and decreased to the basal level after reoxygenation,
with a different rate depending on the treatment conditions. Spectrum fitting
analysis evidenced an alteration of the relative contributions of the endoge-
nous fluorophores, mainly as to both the free/bound NAD(P)H equilibrium and the Flavin amount. The autofluorescence dL has been studied by means of
biochemical assays for tissue damage (the perfuse (LDH leakage and lipid
peroxidation) and to tissue histological patterns. Work is in progress to mon-
itor metabolic changes in the liver graft over time during transplantation in
a pig model. (MIUR-COFIN2001; CNR Special Project: "Biotecnology").
P126
The importance of spectral fluorescence measurements during fluorescence
bronchoscopy in diagnosis of early cancer of larynx and bronchus
N. Boulagouak (Zharhakov)1, V. Sokolov2, E. Filonenko2, Telegina2

It is known that both autofluorescence and 5-ALA-based fluorescence bron-
choscopy are sensitive modalities in detecting of early lung cancer while the
specificity of both methods is relatively low. This paper presents the approach
which is based on combination of endoscopic fluorescence imaging and in
situ spectrophotometry. Fluorescence bronchoscopy were performed with D-
Light / AF System for Bronchoscopy, (Karl Storz GmbH, Germany). For registra-
tions of in vivo fluorescence spectra specially designed medical spectrometer
“Spectr-Cluster” (Cluster Ltd., Russia) was applied. The ratio spectral diagnos-
tic parameter calculated in real time was used for quantification of imaging
and verification of findings received during fluorescence bronchoscopy. 25
patients were examined with the aim to detect occult intraepithelial lesions of larynx and bronchus. The results shown that combination of endoscopic
fluorescence imaging (autofluorescence and S-ALA induced PPIX fluorescence)
with in situ spectral measurements really improves the ability to local-
ize precancerous and early cancerous lesions. The designed approach allows
to also to minimize the number of false-positive results and to reduce the num-
ber of biopsies which are commonly used in search for occult neoplastic
lesions under conventional WLB. The fluoroscopic technique has the poten-
tial to detect the occult foci of malignancy located in sinonasal lumen of larynx or bronchus.

P127
Simultaneous optical and gamma ray tumor imaging in small animals
G. Roberti1, M. Audero2, R. Cozzolino3, P. Lascetti4, R. Lizzi5, G. Mettivier1, P. Ricci6

In the last years the technological development in small animals multimodal
imaging has given rise to a growing and strong interest. We realized an imag-
ing system by HP fluorescence allowing a simple integration with an high
resolution gamma radiolocalization system. The optical and experimental
apparatus uses, as an excitation source, a frequency doubled Nd:YAG laser and,
as a detector, a CCD camera equipped with a cut-on long wave pass filter (cutoff on wavelength=600 nm). Image acquisition analysis were per-
formed with a Hamamatsu Leps 11 system. Tumor detection is enhanced by
high ground digital subtraction between two optimally filtered fluorescence images of tumor region and filtered fluorescence image of an healthy region of the same
laboratory animal. This apparatus was used in conjunction with a gamma emitting
source which used a small area hybrid pixel gamma radiation detector based on the Medipix ASIC read-out technology. High spatial resolution in radioimaging (FWHM < 1 mm) is achieved with a pinhole
tungsten collimator. These two techniques were used to image tumors with
different malignancy (ARO or NPA thyroid carcinoma) implanted in mice. It was shown that, in the optical and gamma ray images of the same mouse,
tumor regions strongly coexist.
PI28
Visualization of the conduction system of hearth by fluorescence spectroscopy
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The conduction system of hearth plays a distinguishing role in normal functioning of a heart. Damaging of conduction tissue during surgery might cause severe conduction disturbances and impairments of the heart cycle. In order to reduce the risk of such accidents it is necessary to find the most convenient way for the visualisation of the conduction system. As the best non-invasive investigation method usually serves optical spectroscopy in recent years, various spectroscopic methods, involving the analysis of the emission and absorption spectra, have become available to study tissue composition and to discriminate between healthy and pathologic tissues with enough good results. UV-visible and infrared absorption and autofluorescence measurements have been employed for spectroscopic characterization of heart tissues. It seems that differences in absorption between the specimens of conduction system and myocardium tissues are mainly determined by the presence of various amounts of aromatic amino acids. The main differences in fluorescence spectra of conduction system and neighbouring tissues were observed in the spectral region of 400–500 nm under excitation at UV spectral region (250–360 nm). It was used for visualization of the conduction system by means of fluorescence microscopy technique.

PI29
Noninvasive method for diagnosis of pigmented skin lesions
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The goals of this work were differentiation between normal skin and pigmented skin lesions by the methods of optical reflectance spectroscopy. Optical reflectance spectra in the wavelength range 400–900 nm were obtained from malignant and benign skin lesions. Characteristic differences in spectra between benign and malignant lesions were studied. Initially lesions were classified dermatoscopically (MoleMax II, DERMAL Instruments). All suspicious lesions were excised. After excision the material was investigated histologically.

The reflectance set-up consists of a light source - halogen lamp (400-900 nm, 500 W), an optical fiber, and a microspectrometer (IPC2000, Ocean Optics). A computer that allows data storage and spectral display controls this system.

Good correlation was obtained between the histological analysis of the patient’s skin and the data from the reflectance spectra. An algorithm for differentiation and valuation of the condition of the skin tissue was created – dimensionless ratios of the intensities of the reflectance signal at 500, 575 and 700 nm of normal skin and pigment lesions, which allows lesion determination by using the unnormalized reflectance spectra – R1=Inorm(500)/pigment(500) and R2=Inorm(500)/pigment(575)/Inorm(575)/pigment(500). These ratios were found to have a definite diagnostic potential, due to the significant difference between benign and malignant pigment lesions.

PI30
Molecular biological investigations of Hypericin polyvinyl-pyrrolidon complexes as proliferation selective marker in bladder cancer cells using Fluorescence correlation spectroscopy (FCS) and NMR-spectroscopy methods
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Supramolecular chromophor-polymer complexes between perylen quinone hypericin and poly-N-Vinyl-amiide are presented as new potent markers of proliferating tissue especially for bladder wall carcinoma cells. Preclinical studies demonstrate significand targeting potential proven by cystoscopy and histology. High postmitotic affinity of hypericin-Polyvinyl-Pyrrolidon (PVP) solutions to intercellular misbodies are investigated by fluorescence correlation analysis (FCS). A correlation between Actin-Musosin activity during the cellcycle and predominant fluorescent marker localization is demonstrated. Furthermore nonplastic cell survival, which is promoted by Protein-Kinase C triggering, can be identified by inhibition of PPK using Hypericin preparations. Fluorescence quantum yield of the water soluble Hypericin-PVP complex solutions is enhanced by decrease of PH. Tumor tissue, which may promote or inhibit the viability of the cells gradually decreased with increasing light dose. However, with 4j/cm2 50% of cells were still viable after 24 hours. Analysis by flow cytometry revealed that a subpopulation of these cells had significantly elevated the surface density of MHC class-I molecules (fluorescence intensity approx. 5fold over that of untreated cells). These findings suggest that repetitive PDT might facilitate CTL-mediated apoptosis of tumor cells and might, therefore, synergize with immuno-therapeutic approaches for at least some tumors.

PI31
Photodynamic Treatment Upregulates MHC Class-I Surface Expression of B16 Melanoma Cells
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University, Salzburg, Austria.

Indigenous antigenic peptides are presented in the context of MHC class-I molecules on the cell surface for recognition by CD8+ T lymphocytes. Downregulation of MHC molecules is a frequently observed strategy of tumor cells to escape immune attack. E.g., B16 melanoma is characterized by extremely low MHC-I surface expression and high tumorigenicity in syngeneic mice. Generally, the efficiency of photodynamic therapy is low for melanotic tumors. On the other hand, PDT has been shown capable of inducing anti-tumoral immunity. Therefore, we investigated the effect of PDT treatment in vitro on the MHC class-I surface expression of surviving B16 cells. When sensitized with 50ng/ml hypericin and then irradiated the viability of the cells gradually decreased with increasing light dose. However, with 4j/cm2 50% of cells were still viable after 24 hours. Analysis by flow cytometry revealed that a subpopulation of these cells had significantly elevated the surface density of MHC class-I molecules (fluorescence intensity approx. 5fold over that of untreated cells). These findings suggest that repetitive PDT might facilitate CTL-mediated apoptosis of tumor cells and might, therefore, synergize with immuno-therapeutic approaches for at least some tumors.

PI32
PDT-treated Dendritic Cells Retain their Potential for Antigen Uptake, Processing and Presentation and T-cell Stimulation
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University, Salzburg, Austria.

The effect of photodynamic therapy (PDT) on anti-tumoral immune reactions is still discussed controversially. Several studies have demonstrated that PDT is able to activate immune reactions against tumor antigens. However, there is also evidence that PDT exerts immunosuppressive effects. Dendritic cells (DC) are professional antigen presenting cells and play an important role in, both, the induction of immune reactions as well as the induction and maintenance of immunologic tolerance. Therefore, we investigated the effect of hypericin-mediated PDT on the capacity of bone marrow-derived DC for antigen uptake, processing and presentation to CD4+ T lymphocytes. Using beta-Galactosidase as model antigens we found that, under sublethal PDT conditions, antigen is still incorporated and degraded by surviving DC. PDT-treated DC, in the presence of beta-Galactosidase, were still able to re-activate splenic T cells from immunized but not from naive mice. Similarly, naive allogenic T cells were activated in an antigen-independent manner by PDT-treated DC, albeit at lower efficiency as compared to untreated DC. Based on these data, we hypothesize that DC localized in PDT-treated tumor lesions could play a role in the regulation of anti-tumor immune reactions.

PI33
Changes of humoral immunity after exposures of volunteers to polychromatic (480-3400nm) light at a therapeutic dose
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Institute of Cytology of Russian Academy of Sciences, St.Petersburg, Russian Federation.

Investigation of immunomodulatory effects of visible + infrared polarized (VIP) light of Swiss phototherapeutic device “Biotron” (480-3400 nm, 95% of polarization, 12 J/cm2 involved turbidimetric determination of Ig M, A, G and standard technique study of immune complexes (IC) in serum of volunteers after a single and a course of 4-9 daily exposures of their sacral area (254 cm2). A statistically significant increase was observed in the levels of IgM, D E and 24 hr after the 1st exposure of volunteers as well as after 4-9 daily phototherapeutic sessions. These changes were of regulatory character, reversely depending on the initial IgM level. IgA level showed a significant increase only on 10th day, while IgG content did not changed. Regulatory influence of VIP-course was also observed in the case of IC content, decrease of this parameter (on average by 15%) being predominant on the 5th day. The data obtained bear witness to prognostically favourable impact of VIP-light treatment on parameters of humoral immunity.
P134
Photopatch testing. A retrospective study
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First systemic photosensitivity reactions were observed after the introduction of sulfonylamides into clinical therapy in the 1930’s. In the 1960’s and 1970’s, numerous photostimulated reactions towards antiseptics were reported in Great Britain and Denmark. Photopatch testing was well-known but standardization was lacking. Scandinavian countries first established test protocols, followed by the German speaking countries. In the 1970’s musk ambrette was the most frequent photosensitizer, sunscreens in the 1980’s. The aim of this retrospective study was to investigate the pattern of photosensitivity reactions seen at our dermatological department, especially to look for possible new photosensitizers.

In the time period from 1981 to 1999, in all 210 patients (145 female and 65 male) with suspected photosensitivity reactions were photopatch tested. Testing was performed with the Scandinavian Photopatch Test Research Group standard procedure, and with the sun screen series. In all, 108 patients (79 female, and 29 male patients) showed 207 positive test reactions (110 photocontact and 97 contact reactions). Cosmetics, sunscreens, and antimicrobials were among the substances most often causing a positive photopatch-test reaction.

P135
Expression and regulation of cytoprotective genes by ultraviolet radiation in skin of patients with psoriasis
University of Dundee, Dundee, United Kingdom.
We have investigated whether inter-individual differences in the cutaneous expression of cytoprotective genes contribute to variability in sensitivity and response to controlled exposure to ultraviolet radiation (UVR) in patients with psoriasis. We have used real-time quantitative RT-PCR to characterise the expression of genes including cytochrome P450s (P450s), glutathione S-transferases (GSTs), drug transporters and stress response genes, in psoriasis patients about to commence phototherapy (n=29). Skin biopsies were taken from buttock sites: (a) 24 h after irradiation with a solar simulator (1-4 x MED site) (b) untreated psoriatic plaque and (c) an adjacent non-lesional control site. UVR significantly induced the expression of the stress response gene cyclooxygenase-2 (median 4.6-fold, range 0.14-22.6) and lead to more modest (2-2.5-fold), or induction of glutathione peroxidase, GSTPI and the drug transporter MRPI. In contrast, GSTP3 (3.36-fold, 1.3-33.3) and MRPI (3.53-fold, 1.3-24.8) were significantly increased in psoriatic plaque, as were P450 CYP2E1 (3.45-fold, 1-28.9) and heme oxygenase (8.43-fold, 2.9-49.7), implying a global up-regulation of drug metabolism in lesional psoriatic skin.

We found considerable inter-individual variation in constitutive cytoprotection of keratinocytes in psoriasis patients. We are attempting to characterise individual patient phenotype using real-time RTPCR and to determine the expression of these genes in response to controlled exposure to ultraviolet radiation (UVR) in patients with psoriasis.

P136
A Comparative Study between Ruby, Alexandrite and Diode Lasers in Treatment Of Hirsutism
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Hirsutism is a common problem for which laser is being the treatment of choice. Several lasers with different wavelength, pulse duration, energy fluences and skin cooling systems are currently used for hair removal. However, the ideal laser parameters and group of patients who respond better to treatment remain largely unknown.

In order to know the ideal candidates for laser hair removal and to evaluate long term efficacy and safety of three different (Ruby, Alexandrite and diode) laser hair removal systems, 171 patients with Fitzpatrick skin types II-IV complaining of hirsutism especially in the chin area were treated after division into 3 groups with these three types of laser. The study showed that all patients displayed reduced and delayed hair regrowth, with better response in younger age and more efficacy and safety in darker skin types with diode laser.

P137
In vivo experimental study of facial nerve repair by diode laser (980nm)welding vs microsurgery, functional and histopathological evaluations
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NATIONAL INSTITUTE OF LASER ENHANCED SCIENCES, Cairo, Egypt.
Regaining nerve function after its injury is still a clinical problem inspite of the great advent of the techniques of nerve repair.nerve repair by laser welding is one of these new techniques,which has many advantages over the microsurgery nerve repair;for this reason, the aim of the present study is to evaluate the effectiveness of diode laser nerve repair from the functional and histopathological points of view.Forty facial nerves from twenty adult mature Valender rabbits were divided into two equal groups. Group A,was diode laser facial nerve coaptation group,and group(B),was microsurgery facial nerve coaptation group.The evaluating parameters included electromyographical, light microscopic, and transmission electron microscopic evaluation.Diode laser nerve repair was found to be more superior over the traditional microsuturing technique for nerve repair at all the evaluating parameters.Diode laser welding was found to be an effective technique for facial nerve repair.It can help for a rapid,accurate,and safe technique for nerve repair at minimal side effects.

P138
Photodynamic effects of zinc(ll)-phthalocyanine on adhesion components of cultured keratinocytes
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The effects of zinc(ll)-phthalocyanine (ZnPc) on cell adhesion have been studied using mouse keratinocytes (Pam-212) and treatment conditions that induced 70% of cell lethality (2 h incubation with 2.5 x 10-7 M ZnPc, followed by 3 min of red light, 21 mW/cm2). After photodynamic treatment the major- ity of the cells tended to be round, gradually losing adhesion between them and with their substrate and finally becoming detached after 24 h. Immunofluorescence showed variable changes in the distribution and reactivity of the adhesion proteins studied, particularly E-cadherin, α- and β- catemins, β1-integrin and vinculin. Immunoprecipitation and Western blot analysis of soluble and insoluble protein fractions confirmed a decrease in the expression of these proteins along time. Concomitantly, actin cytoskeleton suffered modifications in both, the cellular distribution and the reactivity to phallolidin-TRITC. Many photosensitized cells showed deformations on plas- ma membrane and nuclei, with condensed and fragmented chromatin charac- teristic of apoptotic cells. This result was confirmed by the TUNEL assay and by the appearance of the typical DNA ladder pattern in agarose gel elec- trophoresis 24 and 48 h after light exposure. Altogether, the results obtained indicate that these adhesion components of keratinocytes are modified by ZnPc photosensitization.

P139
Photosensitizing properties and photokilling effects of the asymmetric porphyrin CF3 on HeLa cells
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We have studied cell photokilling effects of the asymmetric lipophilic por- phyrin. S-(4-N-(N-2’-dinitro-4’- trifluoromethyl-phenyl)- aminophenyl)- 10,15,20-tris-(2,4,6- trimethoxy-phenyl)porphyrin (CF3) on HeLa carcinoma cells. Treatments were performed using 5 x 10-6 M CF3 (incorporated into liposomes) for 3 or 18h. No evidence of cytotoxicity was found after drug incubation for 18h in the absence of light. In contrast, cells incubated for 18h and then irradiated with violet-blue light (8 mW/cm2) for 7min, showed 8h later a great amount of apoptotic cells (76%). Necrotic cells were observed using 18h treatment followed by 15min irradiation. Cell death mechanisms were identified by staining with toluidine blue and Hoechst 33258, and DNA gel electrophoresis. Scanning electron microscopy was also used to confirm the morphological alterations suffered by apoptotic cells. In addition, treat- ments for 3h followed by 5 or 7min irradiation, induced a mitotic arrest 18h later, increasing the mitotic index from 4.5% to 21% or 25%, respectively. A 32% of mitotic cells were metaphases with disorganized spindles. 48h after this metaphase blockage, 64% were apoptotic cells. Taking into account that apoptosis, necrosis, and mitotic arrest are important issues in the mechanism of cell photokilling, CF3 could be a valuable photosensitizer for research in PDT.
NO had a concentration-dependent cytotoxicity against melanoma cells in experiments with melanoma cells. The results suggest that photogenerated NO is released from the cells, attenuation of the heat shock response, and abrogation of the protective effect.

Cells (A431, normal human epidermal keratinocytes) were exposed to 42°C for 24 hours for 4 consecutive days. HS72 was determined prior to and after each heat shock by EUUA. Immediately after the first and the last heat shock cells were exposed to UVB and survival was determined 24 hours after exposure.

We found that (i) repetitive heat shock has no influence on cell growth; (ii) HS72 is strongly induced after heat shock and is still above baseline at 24h; (iii) re-exposure leads to a superinduction without attenuation of HS72-induction of its UVB-protective effect.

The photochemical decomposition of the metal nitrocompound into medium in a dose-dependent manner. Furthermore, we investigated the influence of Roussin's black salt on the growth of melanoma cells: mouse (S91) and human (SKMel-188) line. Cells were treated with Roussin's black salt (RBS) for 30 minutes in the presence of the viable cells in the measuring box and level of the photoinduced NO donor (RBS) was measured.

We have investigated the influence of Roussin's black salt on the growth of melanoma cells: mouse (S91) and human (SKMel-188) line. Cells were treated with Roussin's black salt (RBS) for 30 minutes in the presence of the viable cells in the measuring box and level of the photoinduced NO donor (RBS) was measured.

Repetitive heat shock does not lead to accommodation in human keratinocytes and protects from UVB-induced cell death

Tetra cationic Phthalocyanine as a potential skin-photosensitizing agent

Four recent results from our laboratories led to the development of a topical formulation for a selected Zn(I)-phthalocyanine which allows the penetration of sufficiently large amounts of photosensitizer into the epidermal layers so that an extensive photoresponse is induced upon visible light irradiation of the phthalocyanine-loaded area.

In particular, we treated dorsal skin of healthy mice with a gel formulation containing 1 mM Tetra cationic Phthalocyanine toward three skin-derived cell lines such as fibroblasts and keratinocytes.

We investigated the influence of Roussin's black salt on the growth of melanoma cells: mouse (S91) and human (SKMel-188) line. Cells were treated with Roussin's black salt (RBS) for 30 minutes in the presence of the viable cells in the measuring box and level of the photoinduced NO donor (RBS) was measured.
P146 Interaction of sulfonated anionic porphyrins with HIV glycoprotein gp120 and associated photodamages
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The glycoprotein gp120 allows cellular entry of HIV and is an attractive target for prophylactic approach involving photosensitization. We consider anionic porphyrins bearing two sulfonate groups and two carboxylic chains that are more or less esterified modulating their overall negative charge. Their ability to inhibit binding of two antibodies of gp120 in the dark or after light illumination is investigated by ELISA. The antibodies are directed toward positively charged epitopes on the V3 loop and the C5 region. The porphyrins bind reversibly to the V3 loop, the highest efficiency being displayed by the esterified compounds. No effect of light is found. In contrast, although no dark interaction between the porphyrins and the C5 region is observed, the binding of the anti-C5 antibody is strongly inhibited upon light irradiation. Again, the esterified compounds are the most efficient. Porphyrin monomers are identified as the photosensitive forms despite the presence of large excess of dimers in the incubation solution. It is suggested that porphyrins bound to the V3 loop could exert photodamages at some distance, in particular within the C5 region that contains several photosensitive amino-acids. The feasibility of altering residues that are not exposed may offer new ways for drug design.

P147 Photosensitization of yeast Candida and Saccharomyces with chlorines
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The recent attention to yeast photosensitization is stimulated by the increasing resistance of pathogenic species to drugs and the need for alternative therapeutic methods. The photosensitization of Candida species, about 10% of which are pathogenic or opportunistic, is of particular interest. We studied the efficiency and mechanisms of photodynamic yeast inactivation in the presence of water soluble photosensitizers - chlorines e6, p6, and their derivatives. All chlorines showed high efficiency (at short incubation time) in the yeast inactivation, 3-formyl-3-devinylchlorin p6 with red absorption near 690 nm in combination with diode laser being the most active in photosensitization of cultures with high density. All chlorines tested had extracellular localization and photosensitization was due to singlet oxygen generation. The inhibition of budding against the continued cell metabolism was typical for the photosensitized cells. The experiments with wild and deficient in the C5 region that contains several photosensitive amino-acids. The feasibility of altering residues that are not exposed may offer new ways for drug design.

P149 Antimicrobial PDT against MRSA S. aureus - a therapeutic window in vitro T. Maisch1, M. Szejtli1, B. Lovel1, C. Abels2;
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Due to increasing antibiotic resistance an alternative to treat skin infections is antimicrobial PDT. However, first photosensitizers (PS) to inactivate bacteria without harming the surrounding tissue in vivo must be defined. Different concentrations (0-100µM) and incubation times (5min, 1h and 4h) phototoxicity of three porphyrin-based PS was determined in primary keratinocytes or fibroblasts. All PS exhibited toxicity against both cell types in vitro at a concentration of 0.1µM to 10µM following irradiation (λmax = 380-480nm; 15.2mW/cm2; 13.76mJ/cm2) after 5min incubation. Sub-cellular localization of the PS was dependent on incubation times: Detection in cytoplasmic membrane after 5min incubation, following longer incubation times, detection intracellularly, very likely in lysosomes according to lysosomes-specific dye. The antimicrobial activity of the PS was investigated using two S. aureus strains MRSA, MSSA and one E. coli strain. Following incubation with PS1 (0.01µM) and PS 2 (0.005µM) irradiation yielded a 3-log step decrease of cell number (n=9.9% of both S. aureus strains and E. coli). This concentrations no toxicity towards fibroblasts or keratinocytes was detected. The results show the first time a therapeutic window in vitro to kill a multi-resistant (MRSA) S. aureus strain by antimicrobial PDT without significant damage to eukaryotic cells.

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Helicobacter pylori infection in the stomach causes peptic ulcers and gastric cancer. Antibiotic therapy leads to 80% - eradication but has side effects and increasing antibiotic resistance. We have discovered that H. pylori is susceptible to killing by visible light. Violet light is most effective (six logs 10 of kill by 0.2-0.2mc2). We propose that the mechanism of killing is the natural accumulation by the bacteria of photoactive porphyrins that produce reactive oxygen species when illuminated. In an animal model, (ferrets naturally infected with H. pylorustan), white light illumination of explanted stomach segments gave 99% bacterial killing. A Phase I clinical trial included patients with dyspepsia and HP infection. At endoscopy a 1-cm2 site in the pyloric antrum was exposed to 405-nm violet light (140-mW/cm2, 40-J), via a diode laser-coupled optical fiber, passed through the biopsy channel. An adjacent site was left untreated. Biopsies were taken from the control and illuminated sites, weighed, homogenized, serially diluted and plated to give CFU/g tissue. Mean control samples had 7.95x10^8+1.69x10^8 CFU/g while treated samples had 7.32x10^7+1.29x10^7 CFU/g representing the killing of >90% of bacteria (2-tailed p<0.0001). Some patients had bacterial killing approaching 99%. Further work is underway to define the utility of this therapy in treating patients.

P151 Synthesis and antiviral activity of cationic porphyrin derivatives against Herpes viruses E. M. Silva1, F. Giuntini1, M. A. Silva1, M. A. Faustino1, J. P. Tomé1, M. G. Neves1, A. C. Tomé1, A. M. Silva1, J. A. Cavaleiro1, J. N. Pegaso1, M. L. Valdeir1;
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Porphyrins, as photosensitizers, are currently finding biomedical application in the treatment of malignant tumors, plaque destruction, psoriatic lesions, and more recently in the treatment of viruses. In particular, herpes viruses are highly disseminated in Nature and they are responsible for a broad range of human diseases, of especial concern in immunocompromised patients, pregnant women and newborns. These facts are important reasons for the search of new antiviral drugs.

The possibility to modulate the required properties of an ideal photosensitizer by modification of the structure or by introduction of specific groups into the porphyrinic macrocycle has been one of our main research interests. In order to look for an effective antiviral agent against Herpes virus, we are studying the synthesis of new cationic porphyrin and chlorin derivatives, and assessing their influence on the replication of herpes simplex virus, types I and II in Vero cells.

The results and experimental procedures will be shown and discussed. Acknowledgments: Thanks are due to FEDER and FCT-project POCI/3875/F/C/F/2001, and also Universities of Lisbon and Aveiro for funding. Some of us are also grateful to FCT for grants (E.M.P. Silva and L. Pegaso for a “BIC”, A.M.G. Silva, J.P. Tomé and F. Giuntini for post-PhD grants).
P152
Photodynamic inhibition of Fusarium spores germination
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Protection of corn cultures seeds against fungal diseases is based nowadays mainly on fungicides. But with a prolonged use of pesticides, pathogens tend to develop resistant forms. Therefore, there is an urgent need for new technologies. As an alternative to fungicides the use of biophysical methods seems to be promising. We have studied the fungicial activity of photodynamic action on asexual spores (conidia) of Fusarium poae. After suspending in distilled water dormant conidia rapidly hydrated and through 2-3 hours the growth of germ tubes began, through 24 hours the branching of tubes and formation of mycelium were observed. If dormant conidia were suspended in solution containing photosensitizers (hematoporphyrin $2 \times 10^{-5}$ M or protoporphyrin IX $5 \times 10^{-6}$ M) and after 1 hour irradiated by visible light ($200 \, \text{W/m}^2$), no growth of the mycelium was not formed. Through 24 hours the lysis of conidia began. Our results demonstrate that photodynamically active exogenous porphyrins can photosensitize the inhibition of fung spore germination; block the germ tube elongation and mycelium formation. These results indicate a perspective for development of new photodynamic technology against fungal diseases. The present work was supported by the Fund of Fundamental Investigations of Belarus.

P153
Putative origin of albinism in androgenetic plantlets in barley (Hordeum vulgare L.)
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Production of albinos is the principal limitation for the production of doubled haploid plantlets of cereals by androgenesis. Absence of chlorophyll may be directly linked to the chlorophyll biosynthetic pathway or be a consequence of other deficiencies. 77K fluorescence spectroscopy revealed that the albinos leaves of androgenetic plantlets do not contain chlorophyll and/or protochlorophyllide. Albinos leaves fed with this compound accumulated protochlorophyllide. Therefore chlorophyll deficiency in regenerated leaves is primarily due to the incapacity to synthesize delta-amino-lavulenic acid. Under a 440nm excitation wavelength, the spectra presented a single band peaking at 634nm, reflecting the presence of nonphotoactive protochlorophyllide. The broadness of the band suggests that several spectral forms of nonphotoactive protochlorophyllide contributed to this band. This was proved by the shift in the position of the emission maximum when changing the excitation wavelength from 440nm to 460nm. The results are discussed in term of the formation of the large aggregates of nonphotoactive protochlorophyllide.

P154
Visible and ultraviolet light stress in cyanobacterium Synechocystis 6803
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We have studied the mechanism of photodamage by visible and ultraviolet light in the cyanobacterium Synechocystis sp. PCC 6803, as well as the interaction of the two spectral regions. Our results show that UV-A and UV-B photons inactivate PS II electron transport at the Mn cluster of water oxidation. This mechanism is independent from the visible-light-induced photoinhibi- tion, and the two spectral ranges do not interact at the level of PSII damage; in intact cells interaction of the two spectral regions results in synergistically enhanced protein repair capacity, which provides protection against photodamage when UV-B radiation is accompanied by low intensity visible light. However, this ameliorating effect becomes insignificant at high light intensities characteristic of direct sunlight. Ultraviolet light damages also the phy- cobilisomes. This effect proceeds more slowly than the inactivation of PSII electron transfer and can not be repaired by rapid protein synthesis. The gen- eral UV response of Synechocystis 6803 cells will also be discussed, based on the data obtained with a DNA microarray containing about 400 stress-related genes.

P155
Fluorescence emission spectroscopy of single chlorosomes lacking bacterio- chlorophyll-a as an energy acceptor
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In an antenna complex (=chlorosome) of green photosynthetic bacteria, self-aggregates of bacteriochlorophyll-BChl-c, d, or e are not formed by a galac- tolipid monolayer where BChl-a protein complex (=baseplate) is located. BChl-a in a baseplate accepts excitation energy harvested by BChl self-aggre- gates, and emits a fluorescence around 800 nm. Here we examined the fluo- rescence emission properties of single chlorosomes lacking baseplates, and compared the fluorescence spectra of single intact chlorosomes. Chlorosomes were isolated from four kinds of green photosynthetic bacteria, and baseplates were removed by treatment with alkaline media. The fluores- cence properties of single chlorosomes were measured with a spectroscopic built into a total internal reflection fluorescence microscope by excitation of BChl self-aggregates. Fluorescence from BChl-a in baseplates could not be observed from all the single alkane-treated chlorosomes, indicating that BChl-a as an energy acceptor was completely removed. Fluorescence bands from BChl self-aggre- gates of single alkane-treated chlorosomes were similar to those of the cor- responding intact chlorosomes. Peak positions in single alkane-treated chlorosomes were also distributed similarly to those in single intact chloro- somes. These show that alkane-treatment of chlorosomes does not influ- ence BChl self-aggregates inside chlorosomes at the single-unit level.

P156
Self-assembly of Zinc Analogues of Bacteriochlorophyll-c in an Aqueous Medium with Phospholipids
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In a chlorosome of green photosynthetic bacteria, a large number of bacteri- ochlorophyll-BChl-c molecules self-aggregate to form rod-like oligomers sur- rounded with a monolayer of lipids and some proteins. Synthetic zinc chlorin possessing a 3-hydroxymethyl group was prepared as a model for BChl-c, which self-aggregate to form a chlorosome-like oligomer. Here we report self-assemblies of synthetic zinc chlorins in an aqueous phos- pholipid solution. Zinc chlorins possessing 6-hydroxypyroxyl or 10-hydroxyde- cycyl chains as a long esterified group at the 17-position were prepared. A methanol solution of the synthetic zinc chlorin and lecithin was diluted with a large volume of water to form pigment – lipid assemblies. Visible absorp- tion and CD spectra showed the synthetic zinc chlorins self-aggregate in a hydrophobic core of lecinthin assemblies. In addition, the long esterified alkyl chain and the terminal hydroxyl group affected the suprastructures of zinc chlorin self-aggregates. The present study suggested that the esterified alkyl chain at the 17-position of the zinc chlorin might intercalate into lecinthin to stabilize the pigment – lipid assembly.

P157
Self-assembly of synthetic bacteriochlorophyll-f analogue having C8-formyl group
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Bacteriochlorophyll-[BChl-f]-c/d/e are known to be major pigments con- structing the main light-harvesting antennae of photosynthetic green bacte- ria. BChl-f has not been found yet, although the name is reserved for the C7- formyl derivative of BChl-d. As a model compound of BChl-f, we have already reported the synthesis and self-assembly of zinc methyl bacterio- phosphorufine (Zn-MBPhe) (1). Here, we report synthesis of 3'-epimerically pure Zn- MBPhe-f analogues possessing the C8-formyl group (2) and their visible absorption, circular dichroism, and fluorescence spectra. The C8-formyl group in 2 cause drastic spectral changes both in monomeric and oligomeric forms compared to the spectra of 1 having the C7-formyl group. For example, self- aggregates of 2 showed more red-shifted $Q_b$ peak in 679THF-H$_2$O than 1, and larger diastereomeric control in the oligomeric $Q_b$ peaks was observed for 3'R/S-2 than for 3'R/S-1.
Influence of 6-benzylaminopurine on chlorophyll accumulation in local sites of greening etiolated leaves of barley

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The kinetics of the chlorophyll (Chl) accumulation in local sites both of senescing (8 days) etiolated leaves of barley and of the same leaves treated with 6-benzylaminopurine (BAP) was studied by employment of a procedure minimizing differences in the extent of development of the plastids. It was shown that the process of greening of senescing etiolated leaves occurs by alternating of the periods of the Chl accumulation with those of the Chl destruction. The kinetics of the Chl accumulation in these leaves acquires of an undulated character. The treatment of senescing leaves with BAP resulted in disappearance of periods of the Chl destruction and the kinetics of Chl accumulation acquire the stepwise character. It is also observed a decrease of the duration of the slowing down periods in the Chl accumulation during the stage of early greening with a simultaneous increase of those in the middle of greening. The periods of the Chl destruction are assumed to be connected with a deficiency of Chl-accepting proteins of photosystems. It is known that BAP stimulates the synthesis of Chl-binding proteins. This appears to prevent the destruction of Chl molecules. The nature of the discrete Chl accumulation and its biological meaning are discussed.

Cadmium toxicity and iron deficiency in poplar leaves detected by fluorescence imaging

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Cadmium is known to influence the iron metabolism of plants, which may play an important role in the biogenesis and functioning of the photosynthetic apparatus. Therefore, photosynthetic parameters of poplar plants grown hydroponically in the presence and absence of Cd (10 mM) and Fe supply (Fe-EDTA, 10 μM) were compared by fluorescence analysis and biochemical methods. We obtained evidence that cadmium induced physiological iron deficiency is connected with the development of cadmium symptoms on photosynthesis: (1) Parallel with leaf chlorosis related to the strength of stress, the iron content of leaves decreased both in cadmium treated and Fe-EDTA-grown iron deficient plants. (2) The symptoms of cadmium toxicity were similar to those of iron deficiency. (3) Differences in the greening of iron-deficient plants in the absence and presence of cadmium could be correlated with differences in translocation of iron into the leaves. To reveal the spatial distribution of cadmium and iron deficiency effects fluorescence images of whole leaves were mapped by fluorescence imaging in the blue, green, red and far-red spectral regions. The iron deficiency was a stronger stressor than the cadmium treatment. The iron deficiency caused the strongest symptoms among the veins particularly next to the main vein.

Spatial pattern of low-temperature photoinhibition on thalli of Antarctic foliose lichen species (Umbilicaria antarctica) visualized by a novel approach.

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Sensitivity of leaves to photoinhibition is believed to be more pronounced under full hydration and low temperature. In this study, we exposed Umbilicaria antarctica, a foliose Antarctic lichen species, to 30 min photoinhibitory treatment (1500 μmol photon m⁻² s⁻¹) under 5 °C. The extent of photoinhibition and rate of recovery were monitored as time courses of chlorophyll fluorescence parameters: Fv/Fm, yield of PSII, and quenching coefficients. To visualize distribution of response to photoinhibitory treatment over lichen thallus, we used a FluorCam (PSI, Czech Republic) device and imaging software. The novel approach enabled to distinguish 0.1 square mm spots (pixels) over the thallus and relate the above parameters to each of them. Physiologically-active thallus zones forming irregular star-like shapes located close to thallus center showed high sensitivity to photoinhibition but also fast recovery. Less physiologically-active marginal parts of thallus were less affected by photoinhibition treatment but showed slower rate of recovery. The extent of photoinhibition in sensitivity to photoinhibition were related to growth pattern of lichen thallus with umbilicate anatomy. The results suggest high capacity of photoprotective mechanisms in Uantarctica to cope with low-temperature photoinhibition. This may help the species to survive during the period of Antarctic spring/autumn.

Effect of Clothing Varieties on Solar Photosynthesis of Previtamin D₃ in vitro study

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ViTD₃ plays an important role in the absorption of calcium and phosphorus from the gastrointestinal tract and in the treatment of scurvy, in addition to stimulating the deposition of minerals in the bone, as well as playing a role in the development of osteoporosis. Sun naturally induces vitamin D₃ photosynthesis. This process is very much affected by a number of factors such as age, geographical location, skin colour and clothing. In the present study it was investigated in vitro the effect of clothing on the solar photosynthesis of vitamin D₃. Different fabric samples were tested for the effect of the increased infrared (IR) and ultraviolet (UV) portions of the solar spectrum (7-DHC) to ViTD₃. 7-DHC was dissolved in methanol to give a concentration of 2.6 ± 10⁻⁸ M. Solutions were exposed to sunlight in quartz containers for predetermined periods either uncovered or covered with the fabric samples under test. Changes in the concentrations of 7-DHC and the photoproducts were monitored by HPLC. Fabrics were graded, as the number of threads per square inch and their sunlight attenuation was determined. 7-DHC is transformed to previtamin D₃ upon exposure to sunlight and the molecular mass of the photoproducts generated was similar to that of 7-DHC. Previtamin D₃ was only detected when treated with fabrics that blocks sunlight, the higher the attenuation was produced. Clothing plays an important role in attenuating sunlight thus leading to diminished ViTD₃ production to an extent that require dietary compensation.

Monte-Carlo computational simulation of energy, structural and photochemical properties for the biogenic amines (serotonin) molecules in solution

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Monte-Carlo (MC) computational simulation of energy, structural and photochemical properties of the biogenic amines (serotonin-ST) molecules in the water solution (cluster). Ab initio quantum chemistry, namely the Monte-Carlo method, can provide the first studies of the important biological interaction and systems. ST (5-hydroxytriptamine,5-HT) appears by means of the hydroxylation for the essential amine acid of the triptophane. Obtained on means of quantum calculations on the basis of programs. The molecule geometry of the solute together with additional data are taken and presented from. The water-water interaction potential action potential was obtained by Matsouka etal from configuration interaction calculation. This potential was used in the MC calculation of the water and found to reproduce the experimental results for energy and structural properties. The bio-molecule-water interaction potential action potential was obtained in the SCF-ICAO-MO approximation and fitted with an analytical function. Calculations were carried out at T=300K and all molecules generated as rigid. Results for potential energies are following in (kcal mol⁻¹): Water-water (neutral molecule) -27.7±0.8 and (zwitterion) -28.0 ± 0.8, the ST-water (neutral molecule) -59.5 ± 2.0 and (zwitterion) -34.8± 1.5. The MC result for bulk water (with the same interaction potentials) is 35.6±0.6 kcal mol⁻¹. Structural characteristics (radial distribution and orientational correlation functions) are also calculated. The zwitterion appears to be strongly favoured with respect to the neutral molecule. Using the selective two-staged (IR+UV) and multi—photon (IR) laser field action method for biological molecules in a solution is connected with known difficulties. General theoretical consideration of using the IR+UV two-staged photo-excitement method with excitement of relatively isolated vibrations which have not strong Fermi resonances for the electronic chemistry of PG in water solution. Besides, it is studied a possibility of selective excitation of vibrational levels by means of the Raman process in a field of the two-frequency visible laser radiation.
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The Photoxicity of Aged RPE Melanosomes  
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This study aims to determine the phototoxic potential of melanosomes isolated from human RPE cells from donors above 60 years of age. Aged RPE cells were fed equvalent numbers of melanosomes from human donors (old >60yrs and young - 20-30yrs) and bovine eyes and maintained in basal medium for 14 days. Cultures were either maintained in the dark or exposed to “blue” light (390-550nm, 2.8mW/cm2) at 37°C for up to 96 hours and assessed for alteration in cell morphology and viability. Melanin content was quantified by EPR. The melanin content ingested by RPE cells was similar for human and bovine melanosomes. Following exposure to both aged human melanin and light, RPE cells demonstrated an abnormal morphology with cells absent from the monolayer. Overall cell viability was reduced by 60% at 48 hours as compared to human melanin-containing cells maintained in the dark. By contrast, ingested young human melanin only demonstrated a 10% decrease in cell viability at 48 hours. Bovine melanosomes did not exhibit any substantial phototoxic effects. These results confirm that aged intracellular melanin can be phototoxic to human RPE cells and supports a role for melanin in RPE aging and the development of age-related retinal degeneration.

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Evolution of ultraviolet radiation-B (UVR-B) induced cataract in the pigmented rat  
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Methods: Pigmented rat. Also, the cataract which develops is partially reversible.

Results: Following exposures to 25 kJ/m2, and intensity of forward light scattering increased till 125, and 25 hours respectively post-exposure and decreased thereafter. All the exposed lenses exhibited a pattern of diffuse cataract development. Progress and extent of peroxidation of (16:0)(22:6)PC was monitored by laser flash photolysis and electron spin resonance spin trapping. Progress and extent of peroxidation of (16:0)(22:6)PC was monitored by laser flash photolysis and electron spin resonance spin trapping. Following exposures to 25 kJ/m2, and intensity of forward light scattering increased till 125, and 25 hours respectively post-exposure and decreased thereafter. All the exposed lenses exhibited a pattern of diffuse cataract development. Overall cell viability was reduced by 60% at 48 hours as compared to human melanin-containing cells maintained in the dark. By contrast, ingested young human melanin only demonstrated a 10% decrease in cell viability at 48 hours. Bovine melanosomes did not exhibit any substantial phototoxic effects. These results confirm that aged intracellular melanin can be phototoxic to human RPE cells and supports a role for melanin in RPE aging and the development of age-related retinal degeneration.  

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Products of peroxidized docosahexaenoic acid generate reactive oxygen species: possible implications for retinal phototoxicity.  
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Docosahexaenoic acid (DHA) constitutes about 50% of all polyunsaturated fatty acids found in photoreceptor outer segments (POS) and it is also present in retinal lipofuscin—product of incomplete degradation of phagocytosed POS. In this study, photoreactivity of 1-palmitoyl-2-docosahexaenoyl-glycerol-3-phosphopholine (16:0)(22:6) PC was analyzed in liposomes made of the native phospholipid or its peroxidized products and their organic solvent extracts by time-resolved detection of singlet oxygen phosophorescence, nanosecond laser flash photolysis and electron spin resonance spin trapping. Progress and extent of peroxidation of (16:0)(22:6) PC was monitored by measurement of MDA using TBA test. We found that products of peroxidation of (16:0)(22:6) PC photo-generated superoxide anion and hydroxyl radical in organic solvents, the oxidized lipid efficiently generated singlet oxygen, with the quantum yield (0.1-0.25) depending on the degree of the lipid peroxidation. Laser flash photolysis measurement of peroxidized (16:0)(22:6)PC revealed formation of a short-lived transient with absorbtion maximum at 600 nm. The adduct, obtained in vitro was characterized by NMR and MS spectroscopy as a cycloduct of psoralen to vinylene bond of acid (AA<>PSO). Physiological reactions towards additives were monitored by flow cytometry, ty tagged with annexin V (Ann) and propidium iodide (PI). All tests were conducted within the range of UVB/PUVA doses applied in vivo. The additives induced gradually shift from Ann+ to Ann+PI+, double positive cells, when treated with 60 to 160 J/m2 of AA. The adduct, AA<>PSO, induced apoptotic changes at a concentration 2.5 times higher than free AA. Accelerated loss of viability and was observed in the presence of psoralen (1μM) and arachidonic acid (20 to 160 J/m2) after UV treatment up to 7.5 J/cm2. Notably, there were twice as many apoptotic cells in PUVA then in only UVB treated cells. Our results indicate that UV radiation synergistically induces comparable apoptosis level when psoralen or arachidonic acid adducts are present. Thus, it is possible that PUVA-induced apoptosis may proceed in part by a light-dependent pathway born in lymphocyte’s membranes.
Non-destructive assessment of broccoli epidermal flavonoids by chlorophyll fluorescence

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Fruit and vegetables have a limited postharvest shelflife. Before there are signs of over ripening, wilt or rot it is hard to evaluate the health related quality. That is the levels of nutrients and secondary metabolites. Rapid and non-destructive methods to assess the actual quality are needed. Chlorophyll fluorescence has proven to be usable for measurement of the content of UV-absorbing epidermal flavonoids in leaves. However, the method has never before been tested on bulky plant parts used as food. In our experiment, broccoli heads were stored in the cold for 12 days with various light treatments 6h per day (24 h darkness control). By using a reference fluorescence signal excited at 685 nm the non-destructive method worked well for the purpose. The absorption of chlorophyll fluorescence excited at wavelengths 382 nm or 530 nm decreased during storage irrespective of light treatment. The results indicate a breakdown of both UV-A absorbing flavonoids and possibly also anthocyanins.

Flavonoid content in apple skin can be estimated by chlorophyll fluorescence measurements

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Flavonoids are an important group of antioxidants, which is thought to have a beneficial effect on human health. During the last decade extensive research concerning different aspects of flavonoids in food has been executed. Accordingly, a need for rapid, non-destructive methods estimating flavonoid content in food has evolved.

In apples most flavonoids are concentrated in the skin. Flavonoids in apple skin include both red pigments absorbing visible light and colourless compounds absorbing ultraviolet light. Chlorophyll fluorescence (CF) is a photosynthetic reaction commonly utilised for measurements on plant leaves and stem. However, in these experiments CF was measured directly on apple skin with portable fluorometers (UV-A-PAM and PAM 2000). The results demonstrate that comparison of CF excited by three different wavelengths (375, 470 and 655 nm) can be utilised for estimation of flavonoid content in apple skin.

Heat shock inhibits UVA-induced cell death

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The ability of heat shock to inhibit UVB-induced cell death has been well documented in human keratinocytes. In the present study we investigated the influence of heat pretreatment on UVA-induced cell death. The human squamous carcinoma cell line A431 was exposed to heat shock (3 h at 42°C) and subsequently to UVA from a metal halide source (315-390 nm, 40-113 J/cm²). Mutsch Supersun 5000, Germany). Viability, lipid peroxidation and 8-oxoguanine were determined after UVA-exposure by an MIT assay, TBARS measurement, and immunocytochemistry (OxyDNA, Biotrin), respectively. At 24 h after UVA-exposure the survival rate was markedly increased in heat pre-treated cells compared to controls at all dosages. This effect was highest when heat shock was applied 2 h before irradiation, and decreased continuously with increasing intervals. In contrast, heat shock applied 2 h before irradiation had no effect on TBARS and formation of 8-oxoguanine. These results for the first time demonstrate that heat shock is able to inhibit UVA-induced cell death. The observed time course indicates that heat shock proteins might be related to this protective effect. Initial data suggest that the heat related increase of survival occurs independently of lipid peroxidation and antioxidative DNA-damage.
P176  Effects of He:Ne Laser Irradiation on the Activity and Expression of Nitric oxide Synthase and reactive Oxygen species in Human Neutrophils In Vitro. Stimulated Effect on Human Lymphocytes

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A comparison of satellite-derived surface UV irradiances with spectroradiometric measurements performed regularly at Thessaloniki, Greece is presented. The satellite irradiances were used for constructing maps of solar ultraviolet radiation over Europe based on a method that combines the use of a standard radiative transfer code (UVspec) and various sources of information on UV influencing parameters (Verdebout and Groebner, 2003). Total column ozone was derived from GOME, TOMS or TOVS satellites while the cloud optical thickness was estimated using METEOSAT/AVHRR data. Other factors taken into account include tropospheric aerosols, snow cover and surface elevation. The resulting products are maps of surface dose rates and daily doses. The data set covers Europe (12E-32W; 34-74N), daily from January 1984 to October 2002 and with a typically 5x5 km spatial resolution. The consistency of the results is tested by comparison with 10 years of daily erythemal doses derived from spectral measurements performed by two Brewer spectroradiometers at Thessaloniki, during the period 1992-2002.

P177  Ground and excited state interaction of phenalenone with nucleic acids and DNA

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Phenalenone is a water soluble aromatic ketone found in fossil fuels and in their combustion products, which is thought to be responsible for the photo-toxicity of crude oils to microalgae, and that has been shown to be mutagenic and carcinogenic in some conditions. Additionally, many secondary metabolites with a phenalenone skeleton have been isolated from plants and fungi, and their photodynamic activity has been acknowledged in some cases. Using steady-state and time-resolved spectroscopies, the interaction of phenalenone with DNA and nucleic acid model compounds has been investigated, in order to further characterize the photobiological activity of phenalenone-based products. Absorption and fluorescence titration experiments show that ground state phenalenone binds to DNA, and laser flash photolysis studies confirm that this binding remains in the excited state. The yield of singlet oxygen production, \( \Phi_\sigma \), is reduced upon binding to DNA. Despite \( \Phi_\sigma \) for phenalenone is almost unity in water, its ketyl radical has been detected in oxygen free solutions, which adds complexity to its interaction with DNA bases. Guanine-type model compounds, but not DNA, quench phenalenone triplet state by direct electron transfer yielding its radical anion. Its overall photoactivity will be discussed in terms of the competing mechanisms that can occur.

P178  Modification of the stress-induced production of ROS by growth conditions in basil leaves

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Many of the plant phenolics are involved in plant stress response and may also serve as screening pigments against ultraviolet radiation (UV). Certain of these compounds have been shown to have antioxidant properties due to their role in scavenging free radicals and other reactive oxidative species (ROS).

Two lines of Ocimum basilicum were grown under visible light either alone or with an additional, relatively low level of UV-B (280-315 nm). Five-week-old basil plants were given short-term stress treatments such as increased level of UV-B and/or drought conditions. Photosynthetic activity (chlorophyll fluorescence) was monitored during the stress treatments. The effect of UV-B radiation on the accumulation of total phenolics was measured spectrophotometrically. ROS formation was monitored both by chemical assays and EPR measurements. Acclimation to low levels of UV-B during growth enhanced the ability of the plants to withstand a second, more severe stress. This was reflected in smaller loss of photosynthetic yield and lower levels of lipid peroxidation products and ROS in UV-acclimated plants than in non-acclimated controls. The results suggest that in Ocimum basilicum leaves both cultivar and growth conditions were determining factors in the potential protection by plant phenolics against effects of drought and enhanced levels of UV-B radiation.
P183 Experiences with in vitro evaluation of sun care products
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The SPF determination in vivo bear some disadvantages such as ethical problems, seasonal dependence, long radiation time, which is time-consuming and expensive. In vitro methods such as the Diffey-Method, have been established. A new instrument for measuring the SPF will be shown. This in vitro method include stability of the sun protection product since it can be measured as the same time as the SPF is determined. The recorded data are evaluated and SPF and the irradiation progress can be determined. This instrument can also be used for the determination of the UVA-protection and UVA-stability. It is possible to specify the absorption values of a sun protection product as mean values of an absorption during the radiation of 1 MED for the UVA in every spectral range between 320 and 400 nm. At the same time, photo-stability during irradiation can be determined. The SPF values of different sun care products, measured in vivo are compared with the in vitro method. The percentage of deviation of the in vitro values from the Colipa values is in the range of 11%. The in vitro determination of the SPF show a satisfactory correspondence with the values which were measured biologically.

P184 Transmittance of sunscreen products from 600 to 800 nm
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The well-known sun protection factor (SPF) that appears on sunscreen products only accounts for the protection afforded up to 400 nm. A photosensitivity protection factor (PPF) has been developed by Moseley et al1 that considers protection up to 600 nm and is therefore relevant to photodermatoses patients. However, photosensitising drugs such as those used in photodynamic therapy provoke photosensitivity above 600 nm. Thus, the transmittance of sunscreens at a concentration of 2 mg cm-2 have been analysed from 600 to 800 nm in order to see if these products will afford any protection in this range. The SPF determination in vivo bear some disadvantages such as ethical problems, seasonal dependence, long radiation time, which is time-consuming and expensive. In vitro methods such as the Diffey-Method, have been established. A new instrument for measuring the SPF will be shown. This in vitro method include stability of the sun protection product since it can be measured as the same time as the SPF is determined. The recorded data are evaluated and SPF and the irradiation progress can be determined. This instrument can also be used for the determination of the UVA-protection and UVA-stability. It is possible to specify the absorption values of a sun protection product as mean values of an absorption during the radiation of 1 MED for the UVA in every spectral range between 320 and 400 nm. At the same time, photo-stability during irradiation can be determined. The SPF values of different sun care products, measured in vivo are compared with the in vitro method. The percentage of deviation of the in vitro values from the Colipa values is in the range of 11%. The in vitro determination of the SPF show a satisfactory correspondence with the values which were measured biologically.

P185 The photosensitisising properties of the sunscreen absorber 2-phenylbenzimidazole-5-sulfonic acid
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The alarming worldwide increase in the incidence of skin cancer has increased public awareness of the dangers of overexposure to sunlight. The topical application of sunscreen products is therefore widely practised to protect both healthy and photosensitve skins from the sun. The filter 2-phenylbenzimidazole-5-sulfonic acid (PBSA) is commonly found in these products as an ultraviolet-B (290-320 nm) absorber. We have investigated the photosensitivity of this absorber and its ability to photosensitise DNA damage when irradiated with those wavelengths of light typically incident on the earth’s surface. A solution of PBSA dissolved in Tris-HCl buffer was found to be photosensitizable. However, when this compound was irradiated at 324 nm in the presence of thymine dimer, it was found to photosensitise the formation of thymine dimer, the well-known precursor to skin cancer. The dimer yields were determined as a function of substrate concentration, photosensitiser concentration and photon flux. A reaction mechanism is proposed for this photochemical process that accounts for the corresponding rate constants. The proposed mechanism has been tested for compatibility with the experimental data by means of computer simulation. The DNA photocleavage properties of PBSA was investigated by means of agarose gel electrophoresis and the fluorescent intercalator displacement technique. PBSA was found to cleave double stranded supercoiled DNA to the relaxed circular and linear forms. A possible mechanism for this photosensitisation is proposed. Although the above results cannot be simplistically extrapolated to human use they nevertheless indicate that there is potential cause for concern.
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