

List of abstracts

1. Photophysics and Photochemistry Sessions

Tuesday morning, basic symposium (Nonell and Ogilby)

Light

Nature and properties of light
Light sources in photobiology
Light conditioning and measuring

Interaction of light with biomolecules

Outcomes of the interaction of light with matter
Light absorption: creating excited states
The properties of excited states
The fate of excited states: radiative and non-radiative unimolecular decay processes
Rate constants, quantum yields, and lifetimes

Basic spectroscopy and Action spectroscopy

What is spectroscopy and what can it do for you.
Absorption techniques: steady-state and time-resolved
Absorption spectra and action spectra
Emission techniques: steady-state and time-resolved
Photothermal techniques

General Photoprocesses Pertinent to Biological Systems

Overview of Unimolecular and Bimolecular reactions
Electron Transfer and Associated Reactions
Energy Transfer and Associated Reactions
Quenching Kinetics in Homogenous and Viscous Heterogeneous Media

Oxygen-Dependent Photoprocesses Pertinent to Biological Systems

General Background on Oxygen: Why is it so unique and important?
Generation of Reactive Oxygen Species (ROS)
Detecting and Characterizing ROS
Characteristic Chemistry of ROS
The Significance of Diffusion Coefficients in Oxygen-Dependent Processes

Wednesday after lunch, special symposium (Nonell and Ogilby)

Measurement, simulation, and analysis of spectroscopic data

Solar irradiance data and the UV Index
Absorption spectra of the aminoacids and nucleobases
Absorption spectra of UV photoprotectors
Absorption spectra of endogenous photosensitisers
Assessing donor-acceptor pairs for Förster resonance energy transfer
Analysis of fluorescence decay data
Analysis of transient absorption data
The singlet oxygen simulator
Analysis of optoacoustic data

Please make sure you have the PhotochemCAD software installed on your computer at the beginning of the course (available at www.photochemCAD.com)

Time- and Space-Resolved Techniques to Study Photoreactions in Biological Systems

Microscopy with Sub-Cellular Spatial Resolution
The Basics of Two-Photon Excitation: The Advantages and Disadvantages

Bibliography

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Brian Wardle, Principles and Applications of Photochemistry, John Wiley & Sons 2009
Nicholas J. Turro, V. Ramamurthy, Juan C. Scaiano, Modern Molecular Photochemistry of Organic Molecules, University Science Books; 2009.
Lars Olof Björn (Ed.), Photobiology, The Science of Life and Light, Springer, 2008
Handbook of Photochemistry, Third Edition (Hardcover)
Marco Montalti, Alberto Credi, Luca Prodi, M. Teresa Gandolfi, Handbook of photochemistry 3rd Edition, Marcel Dekker, 2006
Photobiology Sciences Online, <http://www.photobiology.info/>
J. M. Dixon, M. Taniguchi, J. S. Lindsey, PhotochemCAD 2. A Refined Program with Accompanying Spectral Databases for Photochemical Calculations," Photochem. Photobiol. 2005, 81, 212-213.

2. Biophotonics - light dosimetry in biological tissues

Tuesday after lunch and Wednesday after lunch, basic and special symposia (Wagnieres and Andersson-Engels)

Course description:

The main objective of this course is to convey a broad introduction to the principles governing the propagation of light as well as its interactions with biological tissues, including plants. Consequently, the fundamental optical parameters and the photophysical processes (absorption, scattering, fluorescence) involved in these interactions will be defined and described. Following a brief presentation of the scientific, medical and financial advantages of biomedical photonics, different approach to model the propagation of light will be briefly presented. In addition, the measurement and calculation of the light dose in biological tissues will be described. This will require addressing fundamental concepts in the fields of radiometry and photometry. The instrumentation and techniques used to illuminate or to measure the light in biological tissues will be described. Finally, selected illustrative applications will be presented, including the use of optical and/or spectral techniques in clinical pathology diagnostic.

This course will provide an excellent background to enable the communication and interaction between the students and industrial/laboratory specialists, as well as with medical or clinical partners, thanks to a good understanding of the vocabulary, principles and instruments used in these fields.

In the second part of the “Light dosimetry in biological tissues” more detailed description of some very useful models of light transport will be presented. These include diffusion theory and Monte Carlo simulations. Programs based on these methods will be used to illustrate the distribution of light upon illuminating a turbid media such as biological tissue. Also the time-of-flight response following short pulse illumination will be discussed. Different applications of spatially- and time-resolved measurements will be presented. One application is to use time-of-flight spectroscopy to obtain absorption and scattering spectra of tissues. This information can be used for diagnostic purposes for instance for optical mammography or in brain activation studies, as well as to obtain the optical properties at the activation wavelength in laser therapy allowing accurate dosimetry of this specific treatment. The presentation will also provide examples of state-of-the-art instruments to conduct such measurements and briefly survey the field of research.

3. UV (from DNA damage to human skin)

Wednesday morning, basic symposium (Douki and de Gruijl)

UV-induced damage to DNA: from the initial chemical modifications to repair and mutagenesis (Douki)

Induction of damage to cellular DNA is a major deleterious event in cells and skin exposed to solar radiation. Indeed, modification of the chemical structure of DNA may lead to the blocking of vital biological processes such as transcription and replication. These arrests may result in cell death. In case of survival of a damaged cell, errors are likely to occur upon replication of the damaged DNA templates leading to irreversible mutations in the progeny of the cell. These mutagenic events represent the earliest step of carcinogenesis. DNA damage is also implicated in other adverse effects of exposure to solar radiation such as immunosuppression. Fortunately, cells are equipped with a series of DNA repair systems that are able to restore the integrity of the genome in most cases. Evidence has been obtained for a major role played by the ultraviolet part of the solar spectrum and the type of DNA lesions was found to greatly depend on the wavelength of the incident photons. The effects of UVB (280-320 nm) mostly result from a direct absorption of the radiation by DNA, leading to dimerisation of pyrimidine bases. In contrast, DNA lesions induced by UVA (320-400 nm) are mostly mediated by photosensitized oxidation reactions. The purpose of this session is to present the main chemical modifications induced within DNA upon exposure to sunlight and to provide information on repair and mutagenesis of these lesions.

UV carcinogenesis: early p53 mutations and tumor progression (de Gruijl)

UV radiation causes DNA damage and a plethora of collateral effects in the skin. Acute responses should serve to fend off or cope with direct toxicity, and minimize long term adverse effects, such as skin cancer. Sunburn is an inflammatory reaction caused by cytokine release from injured skin cells, and curiously, it is accompanied by suppression of cellular immunity. After excessive exposure massive cell death (apoptosis) may occur, which after a couple of days results in peeling of the skin (an all too common phenomenon after a sunny day at the beach). Apoptosis of an overly damaged cell will prevent it from contracting mutations, which can lead to cell transformation and carcinogenesis. However, the UV-induced immunosuppression may enhance UV carcinogenesis. As a transcription factor, the p53 tumor suppressor protein induces cell cycle arrest, DNA repair and – in case of an overload of DNA damage – apoptosis. The protein forms an important nodal point in a network of incoming and outgoing stress signals, and its malfunction leads to genomic instability. This Achilles' heel of the cell proves to be mutated in a majority of skin carcinomas, and the mutation spectrum bears the "UV signature". In experimental UV carcinogenesis the skin shows microscopic foci (clones) of cells overexpressing mutant-p53 well before the occurrence of the ultimate carcinomas with similarly overexpress mutant p53, i.e. the foci appear to be microscopic precursor lesions. In contrast to chemical skin carcinogenesis, and other cancers, loss of p53 function appears to be an early event in UV

carcinogenesis. Next to disruption of tumor suppression, oncogenic pathways are likely to be activated in skin carcinomas, e.g., the Hedgehog pathway in basal cell carcinoma and probably the Ras pathway in squamous cell carcinomas. At what stage this happens and precisely how is not yet clear.

Wednesday after lunch, special symposium (Sage and de Gruijl)

Cellular response to solar UV-induced DNA damage (Sage)

The photons of sunlight precipitate a series of genetic events in skin, potentially leading to cancer. Both UVB (280-320 nm) and UVA (320-400 nm) radiation is able to cause a range of damage to biomolecules, including to DNA. If not repaired, DNA damage may generate mutations. DNA damage and mutations represent early genetic events in photocarcinogenesis process. Maintenance of genome integrity is thus essential to minimize heritable mutations and to promote healthy survival of cells, tissues and organisms. In fact, eucaryotic cells have evolved surveillance mechanisms such as DNA repair, cell cycle checkpoints and stress signalling pathways which constitute the DNA Damage Response (DDR). Cell cycle checkpoints, also called DNA integrity checkpoints monitor DNA damage and coordinate cell cycle progression and DNA repair processes. Key mediators of these checkpoint pathways are the Ataxia-Telangiectasia-Mutated (ATM) and ATM- and Rad3-related (ATR) protein kinases. ATM and ATR activation, through a cascade of phosphorylation of mediator and transducer proteins, leads to cell cycle arrest, giving time for repair. If a cell has its genome too heavily damaged because of excessive exposure, DDR, *via* p53 activation, drives the cell towards apoptosis. In addition, the p38 stress activated protein kinase pathway constitutes a third checkpoint pathway in response to genotoxic and nongenotoxic stress. It is well established that checkpoint activation by UVC (and UVB) is mediated by ATR pathway. DNA damage response following UVA exposure has received less attention. Collectively these surveillance mechanisms mitigate the effects of UV exposure.

Photoimmunology, UV and vit.D and UVA signalling (de Gruijl)

As one can gather from the title above, UV has many diverse effects in cells and in the skin. In the morning session we focused on genotoxicity and mutations, which when occurring in *p53* can contribute to skin carcinogenesis. And in the first session this afternoon, we had a closer look at the cellular stress response upon UV irradiation. In this session we will touch upon the wide variety of UV reactions in the skin.

In classic mouse experiments in the 1970s Margaret Kripke showed that UV exposure induced suppression of cellular immunity against UV-induced skin tumors, and not only a transient suppression, but also a lasting UV tumor-specific tolerance mediated by “suppressor T cells” (now re-dubbed “regulatory T cells”, or “T-regs”). Thus, the UV radiation not only causes skin tumors by mutagenesis but also by neutralizing immune defenses. It turned out that UV did not only raise tolerance to UV-induced tumors, but could also induce tolerance to contact allergens if UV exposure preceded sensitization (immunization) against the allergen. Although UVA can clearly induce suppression of cellular immunity, UVB radiation proved to be most effective per J/m². Interestingly, it was later found that UVA radiation could actually counteract the UVB-induced immunosuppression, which was related to UVA’s induction of heme oxygenase, IFN-gamma and IL12 (opposing the UVB-induced IL10). While UVB suppresses the acquired cellular immunity, it also stimulates innate immunity (inducing

antimicrobial peptides), the first and non-specific line of immune defense against microorganisms.

Surprisingly, immunization against a contact allergen could be suppressed by UV exposure in virtually all human subjects tested, given high enough dosages, suggesting this suppression to be a sound physiologic response to UV exposure. Hence, one could infer that in absence of adequate immunosuppression UV radiation might elicit illicit immune responses against the exposed skin (presumably by generating “neo-antigens” in the skin). Indeed, people with a “sun allergy” (Polymorphic Light eruption) turn out to have (slightly) aberrant immune responses to UV with a possible skewing toward pro-inflammatory reactions. By “UV hardening” of the skin these responses can apparently be normalized.

An aspect of solar UV(B) exposure that has recently come into the lime light is the formation of vitamin D₃ in the skin. In 1982 the wavelength dependence of the photoconversion of 7-dehydrocholesterol to pre-vitamin D₃ revealed a peak around 300 nm. Although not intended for that purpose, this “action spectrum” has since been used to ascertain “vitamin D-effective UV dosages”; ignoring the multiple back and forward UV-driven photochemical reactions involved, and the consequential wavelength interactions which are likely to invalidate the premise of additivity on which spectral weighting with action spectra is based. Classically, vitamin D is known to be important for healthy bones, but research over the last decades have shown vitamin D to have a much broader impact on our health. Cells in various organs can convert vitamin D to its final active form, 1,25dihydroxyvitamin D, to locally address cells with vitamin D receptors; in epithelia growth is thus down-regulated and differentiation stimulated. Cancer cells from such tissues are generally still responsive to this vitamin D signaling and may thus be inhibited in growth. Interestingly, vitamin D shares some common effects with UVB, notably the immunosuppressive effect which probably contributes to the therapeutic efficacy of vitamin D analogs on psoriasis (a putative autoimmune disease).

4. Photodynamic therapy – Fluorescence diagnosis

Thursday morning, basic symposium (Jori – Walt)

Phototherapeutic and photodiagnostic approaches for a variety of diseases using photodynamic sensitising agents (Jori)

A number of methods are presently available for the selective or at least preferential targeting of diseased tissues by photodynamic agents. This goal can be achieved through an adequate control of the interplay of different factors, including the physical and chemical properties of the photosensitiser, the mechanisms involved in the transport of the topically or sistemically administered photosensitiser, and the physiological, morphological and biochemical features of the diseased tissue. In general, photodynamic sensitisers bring about their cytotoxic action through the generation of reactive oxygen species (ROS) via energy or electron transfer from their long-lived triplet state; such process is independent of the excitation wavelength. Thus, porphyrins and their analogues (e.g., chlorins, porphycenes, phthalocyanines) represent ideal candidates for the promotion of *in vivo* photosensitisation since they exhibit absorption bands in the blue, green, yellow and red region of the visible spectrum, hence they can be photoexcited by a variety of wavelengths: this results in the illumination of different tissue volumes, thereby allowing one to modulate the depth of the photoinduced tissue damage and to tailor the overall extent of the photoprocess to the characteristics of the disease to be treated.

In general, blue or green light, which has a limited penetration power into most human tissues, can be used for the photodynamic therapy (PDT) of superficial cutaneous pathologies, such as basalomas, acne or bacteria-infected wounds, whereas more deeply penetrating red wavelengths can be more conveniently adopted for the treatment of bulky tumours or thick psoriatic plaques.

Porphyrinoid compounds have the additional advantage that their chemical structure can be engineered by various synthetic pathways, including the introduction of peripheral substituents protruding from the peripheral positions of the tetrapyrrolic macrocycle, the chelation of metal ions with the central nitrogen atoms and the addition of axial ligands to the fifth and sixth coordinative positions of the metal ion. As a consequence, the properties of the porphyrin can be fine-tuned to achieve any desired level of water-solubility, hydrophobicity, affinity for selected subcellular sites, distribution among different tissue compartments. This has important consequences for the interaction of the porphyrin with possible carriers (e.g., liposomes, oil emulsions, serum proteins), as well as for the pattern of its localization in the diseased tissue, hence for the mechanism of the photoinduced damage. At present, PDT is emerging as a promising modality for the treatment of various solid tumours, microbial infections, a large number of skin diseases, age-related macular degeneration and the recanalization of obstructed arteries especially after balloon angioplasty.

Most importantly, most photodynamic sensitisers are endowed with a readily measurable fluorescence emission, hence such sensitisers can be favourably used also as diagnostic tools, e.g. for the detection of early cancer, given the high sensitivity of this spectroscopic technique.

References

“Photodynamic Therapy and Fluorescence Diagnosis in Dermatology” (Eds. P.G. Calzavara, R.M. Szeimies, B. Ortel), Elsevier, Amsterdam, 2001

“Photodynamic Therapy” (Ed. T. Patrice), Royal Society of Chemistry, Cambridge, 2003

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“Photophysical and photobiological processes in the photodynamic therapy of tumours” M. Ochsner, *J. Photochem. Photobiol., B: Biol.*, 1997, 39: 1-18

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“Photosensitised inactivation of microorganisms” G. Jori, S.B. Brown *Photochem. Photobiol. Sci.*, 2004, 3: 403-405

“In vivo fluorescence spectroscopy and imaging for oncological application” G. Wagnières, W.M. Star, B. Wilson, *Photochem. Photobiol.*, 2004, 68: 603-632

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Clinical Photodiagnosis and Photodynamic Therapy applications. (Walt)

A paper published by Raab in 1900, was first to describe destruction of a living organism (paramecium) by the combination of dyes and light¹. The earliest clinical application was published in 1903 by Tappeiner and Jesionek², who used topical and intratumor injection of eosin activated with sunlight or an arc lamp to treat a skin cancer. During the following decades clinical photodiagnosis (PD) and photodynamic therapy (PDT) did not show a continuous and systematic development. Besides some heroic applications early in the 20th century³, regular PDTs started only around 50 years ago. First generation photosensitizers such as Photofrin[®] enabled early PDT approaches in dermatology and were expanded slowly towards other disciplines such as esophageal-, lung-, gastric-, cervical- and bladder cancer⁴. In our days PDPDT is additionally established in neurosurgery, in head and neck cancer and in urology. In the latter case mainly for the localisation of bladder cancer. Countless photosensitizers were developed but only a few got admittance for clinical treatment. Successors of Photofrin were the second generation photosensitizers Visudyne[®], Levulan[®], Foscan[®], Metvix[®] and Hexvix[®] and were developed mostly against cancer but some of them have a focus on very specific treatment modalities in non-cancerous ailments. Visudyne for example is applied for age related macular degeneration which affects older adults by a loss of vision. More recently antimicrobial PDT was selected as a promising topic⁵. PDT for periodontitis is a rapidly growing clinical application and other dental applications are under investigation. Antimicrobial PDT will become more important in the future as antibiotic resistance is only expected to continue to increase⁶. There will be more sophisticated PDs and PDTs in the future e.g. for the treatment of chronic inflammation. In addition, a combination of PDT with targeted nano particles seem to be very suitable to pave the way for considerable improvement of this technology. No doubt, PD and PDT have arrived in many clinical therapy concepts. An actual search on PubMed (<http://www.ncbi.nlm.nih.gov/sites/entrez>) with the term "photodynamic therapy" shows around 13'000 publications, underlining a very positive trend. The newly established European Platform for Photodynamic Medicine (EPPM, <http://www.eppm-photomedicine.org/index.html>) is a further chance to keep an eye on the most recent clinical developments in PD and PDT. Finally, a new journal entitled "Photodiagnosis and Photodynamic Therapy" (PDPDT, <http://www.pdpdt-journal.com/>) is available which covers most of the present clinical applications in the field. PDPDT is the official journal of EPPM offering many synergies for intense networking.

References related to this course

1. Raab, O. Über die Wirkung fluoreszierender Stoffe auf Infusorien. *Z Biol (München)* 1900;39:524-546.
2. Tappeiner, H., and Jesionek, A. Therapeutische Versuche mit fluoreszierenden Stoffen. *Münch Med Wochenschr* 1903;50:2042-2044.
3. Meyer-Betz, F. Untersuchungen über die biologische (photodynamische) Wirkung des Hämatoporphyrins und anderer Derivative des Blut- und Gallenfarbstoffs. *Dtsch Arch Klin Med.* 1913;112:476–503.

4. Dougherty, T. A Brief History of Clinical Photodynamic Therapy Development at Roswell Park Cancer Institute, *Journal of Clinical Laser Medicine & Surgery* 1996;14:219-221.
5. Jori G. Photodynamic therapy of microbial infections: state of the art and perspectives. *Environ Pathol Toxicol Oncol.* 2006;25:505-19.
6. Dai T, Huang YY, Hamblin MR. Photodynamic therapy for localized infections-state of the art. *Photodiagnosis Photodyn Ther.* 2009;6:170-88.

Clinical PD-PDT application in presence of a herbal antidepressant leads to a new interpretation of the photosensitizer hypericin. (Walt, 2nd lecture)

This course is foreseen to bring together clinical reality and basic research. An emergency situation of a patient with breast cancer raised a couple of suggestions for our actual research projects. Previously, we described a patient who suffered from a pronounced phototoxic reaction after being treated with 5-aminolevulinic acid (5-ALA) for intraoperative localization of a breast tumor and its margins. In our clinical trial, 5-ALA was given to 20 patients with palpable breast tumors and 19 thereof had no phototoxic signs. One of the patients, however, presented with burning erythematous rash and severe swelling of the face, neck and hands 6h after 5-ALA administration. It turned out that this patient had been taking a commercially available St John's wort extract, a popular over-the-counter antidepressant preparation containing a considerable amount of hypericin. In a follow-up experiment by using white light, we were able to demonstrate a synergistic phototoxicity between 5-ALA-induced protoporphyrin IX (PpIX) and the above extract, reducing cell survival *in vitro* in the human keratinocyte cell line HaCaT. Hypericin is a naturally occurring hydroxylated phenanthroperylene dione present in St. John's wort extract and is a potent photosensitizer which can be applied locally or systemically and serves as an agent for PDT as well as a marker for the fluorescence detection of malignancy. A combination of 5-ALA and hypericin which was added to the culture media presented a significantly enhanced phototoxicity after illumination with white light on human endometrial cancer cells (HEC-1A). Obviously, both 5-ALA and hypericin based phototoxic mechanisms are turned on in parallel and act synchronically but separately from each other in these cells. Their specific excitation wavelengths (590nm for hypericin and 635nm for PpIX) do not overlap and are included within the white light spectrum.

More recent results on head and neck squamous cell carcinoma cells indicate that a combination of hypericin and Foslipos[®] (a derivative from the photosensitizer Foscan[®] from Biolitec AG, Jena, Germany) may have synergistic phototoxic effects too. Based on fluorescence imaging we found that both photosensitizers were accumulated at different cell compartments and this could well foster an additional PDT effect. The application of mixtures with overall lower concentrations of each photosensitizer may be advantageous compared to established conditions. We expect many more challenging details by further research with hypericin and similar pharmacological combinations.

References related to this course

Ladner DP, Steiner RA, Allemann J, Haller U, Walt H. Photodynamic diagnosis of breast tumours after oral application of aminolevulinic acid. *Br J Cancer* 2001;84:33—7.

Ladner DP, Klein SD, Steiner RA, Walt H. Synergistic toxicity of delta-aminolaevulinic acid-induced protoporphyrin IX used for photodiagnosis and hypericum extract, a herbal antidepressant. *Br J Dermatol* 2001;144:916—8.

Schneider-Yin X, Kurmanaviciene A, Roth M, Roos M, Fedier A, Minder EI, Walt H. Hypericin and 5-aminolevulinic acid-induced protoporphyrin IX induce enhanced phototoxicity in human endometrial cancer cells with non-coherent white light. *Photodiagnosis Photodyn Ther.* 2009;6:12-8.

Friday after lunch, special symposium - Clinical PDPDT (Kostron and Rhodes)

PDD/PDT in Neurosurgery- clinical applications (Kostron)

Scope:

After the lecture the attendees should have knowledge about clinical photodynamic application in Neurosurgery including

- pathological entities
- neuroimaging
- indication
- what sensitizer
- PDD and PDT and combination of both
- which light dose regime
- post treatment care

Outline:

With current treatment methods the prognosis for patients with aggressive brain tumors is dismal with a median survival of 15 months. Treatment failure is usually due to local recurrence of tumor. Intra-operative photodynamic detection (PDD) of tumor tissue and post-surgical photodynamic therapy (PDT) of the resection cavity may be of benefit.

Photodynamic techniques such as photodynamic diagnosis (PDD) , fluorescence guided tumor resection (FGR) and photodynamic therapy (PDT) are undergoing intensive clinical investigations as adjunctive treatment for malignant brain tumours.

At the beginning a short outline of the neuropathological entities will be presented as well as diagnostic tools such as MRI, CT and PET.

This will be followed by basics of photomedicine in neurooncology which differs from the general oncological and non-oncological situation.

A historical review upon the development of PDT will be given.

In the following the indications for PDD and PDT in brain tumors will be discussed.

Due to special anatomical situation of the brain specific features and requirements of the sensitizers have to be fulfilled.

Light and laser technologies for fluorescence guided resection and interstitial light delivery will be presented. Practical examples will be presented.

Furthermore various light dose regimes for high and metronomic light delivery are demonstrated and discussed.

Real clinical cases for practical demonstration will be shown and discussed in detail.

Finally this session will be concluded with an overview on the current clinical data and trials as well as on future developments in detection and sensitizer and light delivery .

PDT in dermatology (L.Rhodes)

The biological actions of topical PDT and how the results of laboratory research may be applied to the clinical practice setting will be presented. Secondly, which skin conditions currently are or may in the future be treated with PDT, and why will be discussed. Areas covered include mechanistic aspects, malignant and non-malignant indications, reasons for selecting this treatment modality, practical considerations, and potential use of PDT as a biological response modifier.

Saturday after lunch, special symposium – Experimental/preclinical (Piette and Berg)

Mechanisms involved in cancer cell killing by Photodynamic therapy (Piette)

Scope

This lecture will give an overview of the complexity of the molecular mechanisms that are involved in cancer cell killing by photodynamic therapy (PDT).

Outline

I will review:

- the importance of reactive oxygen species (ROS), and in particular, singlet oxygen.
- the cellular compartments targeted by ROS
- the cell death mechanisms known to be activated by PDT
 - Apoptosis
 - Necrosis
 - Autophagy
- The cellular defenses against damages caused by PDT
 - Antioxidant defenses
 - Anti-apoptotic molecules
 - Conversion of autophagy into apoptosis or necrosis.
- The role of the immune system in cancer cell elimination after PDT
- The effects of PDT on the tumor vasculature.

Lysosomes and endosomes in PDT: Cellular uptake, photocytotoxicity mechanisms and utilization for drug delivery (Berg)

Most of the photosensitizers used in experimental and clinical photodynamic therapy (PDT) of cancer localize extranuclearly in cells: in the plasma membrane, in mitochondria, in endoplasmic reticulum, in Golgi apparatus and in lysosomes. The intracellular localization is dependent upon the chemical properties of the photosensitizer, *i.e.* its hydrophobicity, charge and amphiphilic character. The identity of the intracellular targets responsible for photochemically induced cell death depends on the parameters mentioned above. The structural requirements for cellular uptake of photosensitizers into endocytic vesicles (endosomes and lysosomes), the cellular mechanisms involved in the photochemical activation of photosensitizers located in endocytic vesicles and the subsequent cell death, and how photochemical targeting of endocytic vesicles may be utilized to deliver drugs to cells (photochemical internalization, PCI) will be described. Alternative targeting and delivery technologies for photosensitizers will also be reviewed.

5. Photosynthesis

Thursday after lunch, basic symposium – photosynthesis (Bassi and Morosinotti)

Photosynthesis (Morosinotto)

This part of the lecture will review of the light dependent reactions of oxygenic photosynthesis and how light is absorbed by photosynthetic complexes and converted into photochemical energy. It will also describe protein composition of photosystems, the molecular machineries catalysing the primary steps of light conversion into the chemical bond energy of organic compounds. In higher plants, algae and cyanobacteria, these steps are operated by two pigment-protein supercomplexes localised in the thylakoids membranes, called Photosystem I (PSI) and II (PSII). Their activity leads to the transport of electrons from the water to a final acceptor with higher potential (NADP+) as well as to a asymmetric protons and charge distribution, which is the motor force for ATP synthesis. The peculiarity of Photosystems with respect to the other chloroplast complexes is their binding of massive amounts of pigmented compounds, chlorophylls and carotenoids.

Photosystems are composed by two moieties: attention will be first dedicated to the core complexes, which are responsible of conversion of light into chemical energy. Later we will describe antenna systems, which are composed by pigment binding proteins responsible of increasing light harvesting capacity. While core complexes are conserved, antenna systems diverged during evolution and differences between different organisms capable of oxygenic photosynthesis (cyanobacteria, algae and plants) will be described. Particular attention will also be dedicated to how proteins of the photosynthetic apparatus are able to modulate pigments biophysical properties.

Photosynthesis (Bassi)

Abstract coming

Friday after lunch, special symposium – photosynthesis (Bassi and Morosinotto)

Photosynthesis (Morosinotto)

This part of the lecture will instead focus on the regulation of photosynthesis light reactions. In a natural environment, light for photosynthetic organisms represents not only an energy supply but also a source of reactive oxygen species, when light absorbed is in excess. Plants are particularly exposed to oxidative stress because they live in a land environment where illumination is generally stronger and oxygen diffusion is faster with respect to water. To avoid cellular damages different photoprotection mechanisms evolved, allowing plants survival in these conditions. Differences with other photosynthetic organisms living in water

will also be reviewed. In particular, we will describe recent findings on the faster among photoprotection mechanisms called NPQ (Non Photochemical Quenching). This is particularly interesting because it allows modulation of light harvesting efficiency within a few seconds without requiring protein synthesis or post-translational modifications. This is a valuable example on how protein conformational changes are able to drastically modulate quantum yield of pigments they are binding.

Photosynthesis (Bassi)

Abstract coming

6. Environmental photobiology

Friday morning, basic symposium and Friday after lunch, special symposium (Bornman and Ghatti)

Linkages between ozone depletion and climate change, and their consequences for photobiological processes (Bornman, Lecture 1)

The decreases in the amounts of stratospheric ozone (the ozone layer found 10 -50 km above the Earth's surface) as a consequence of human activities (mainly production of synthetic chemicals that destroy ozone), has caused concern in the last few decades because of the important UV-filtering role of the ozone layer in protecting life on Earth from damaging UV radiation. In particular, it is the UV-B portion (280-315 nm, as defined by the Commission Internationale d'Eclairage (CIE)) of the sun's spectrum reaching Earth that is most affected by small changes in stratospheric ozone and thus any decrease in this UV-absorbing layer will have an impact on the amount of incoming UV radiation. Consequently, a decreased stratospheric ozone layer can have negative effects on humans, other animals, plants and microorganisms, as well as on structural components, such as materials (e.g. plastics, wood and wool). These effects range from skin cancer and eye damage to decreased productivity in terrestrial and aquatic organisms, to diverse changes in the cycling of nutrients and toxic metals through the different ecosystems. Changes in atmospheric ozone also influence natural climate processes, giving many feedback reactions.

Some of these links address the warming effect on Earth by the greenhouse gases, which by their prevention of some of the re-emission of the radiation back to the upper atmosphere, thus cool the upper atmosphere leading to favourable conditions for stratospheric ozone depletion. Ozone depletion and climate change are linked as physical processes and also through global policies such as the Montreal Protocol and the Kyoto Protocol and its subsequent amendments. The Montreal Protocol, set up to protect the stratospheric ozone layer by phasing out substances that deplete it, the so-called ozone-depleting substances (ODS), has effectively resulted in the control of some 100 ODS. The link here is that some of the ODS are themselves also greenhouse gases, resulting in a significant contribution to the commitments of the Kyoto Protocol, which has focussed on reducing these gases. The increasing global temperatures as a consequence of greenhouse gas emissions coupled with ozone depletion have resulted in complex, sometimes interactive, physical and biological impacts with far-reaching effects for life on Earth. Although the Montreal Protocol has been highly successful, several issues remain as threats to our climate and quality of life. The most important of these are the long atmospheric lifetimes of some of the ODS, estimated from 1,000 to 50,000 years, as is the case for the perfluorocarbons. In addition, some of the substitutes for the most harmful ODS, have also been shown to be greenhouse gases.

Apart from ozone depletion, which is predicted to slowly recover, other factors also contribute to an increased UV irradiance, especially in the Northern hemisphere. These include decreased aerosol concentrations, decreased air pollution and decreasing cloudiness, making UV radiation an important component to consider in the research fields of environmental photobiology.

This lecture will provide an intriguing background of ozone depletion linked to other climate variables that will deepen understanding of the biological consequences of UV radiation and climate change.

Suggested reading

Understanding and responding to climate change. 2008. Highlights of National Academies Reports.

http://dels.nas.edu/dels/rpt_briefs/climate_change_2008_final.pdf

The environmental and ecological significance of UV-B radiation coupled to climate change interactions (Bornman, Lecture 2)

Research on the effects of UV-B (280-315 nm) radiation is increasingly focussed on the modifications to organisms caused by this radiation, with attention to the interactions of different climate variables. This approach has been applied to processes from the molecular to the whole organism and ecosystem levels and has widened our perspective on the responses to multiple stresses, since they are often unique and not immediately indicative of each stress applied alone.

Implications of ozone depletion, consequent elevated UV-B radiation and interactions of climate change factors are posing many unknowns for life on earth. The most significant climate change factors include increasing greenhouse gas emissions, rising temperatures in mid-latitudes, frequent flooding and drought events. These occurrences result in a wide range of consequences for plants and animals, among them shifts in seasons, spreading of vector-borne diseases into new areas, altered herbivory and pathogen attack, changes in species composition and abundance, as well as indirect effects, including impacts on ecosystem processes below soil surfaces.

The key findings for the environmental effects of an enhanced UV-B radiation, whether it is a consequence of a depleted ozone layer or because of changes in other climate-linked processes, include damage to the eyes, skin cancers, suppression of the immune system, and some modification to certain diseases with respect to human health. In addition, the biological availability and toxicity of metals, and alterations in carbon and nutrient cycling in plant and aquatic ecosystems are enhanced by increasing UV-B radiation. The phenomenon of cross-tolerance reflects some of the synergistic relationships among different stresses that may lead to resilience. However, a stressed biological system may show resilience in some situations, but become more susceptible in others.

This lecture will explain some of the mechanisms of response to a changing environment, as well as addressing the negative, useful and modifying effects of an increased UV-B radiation against a background of interacting climate change factors. Examples will be drawn mainly from the terrestrial and aquatic environments.

Suggested reading

Smith, W.K., Gao, W. & Steltzer, H. 2009. Current and future impacts of ultraviolet radiation on the terrestrial carbon balance. *Frontiers of Earth Science in China*. 2009, 3(1): 34–41. (review article).

Roberts, M.R. & Paul, N.D. Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defence against pests and pathogens.

Action spectroscopy for the study of the effects of environmental UV (Ghetti)

Ecologically relevant studies aimed to determine action spectra of environmental UV effects require the careful consideration of various experimental features, such as treatment duration (short exposures may not allow to observe the overall response of the organism), use of suitable samples (*in vitro* studies can have limitations for extrapolation to the performance of the whole organism), presence of reciprocity effects and irradiation patterns.

It should be stressed the importance of using irradiation conditions not too far from present levels of natural radiation or those under predictable ozone depletion scenarios. Using extraterrestrial or so high irradiances that the organisms are irreversibly damaged certainly produce a clear effect, but not so meaningful for a realistic assessment of ozone depletion consequences.

Not only radiation intensity, but also its spectral composition is crucial. Monochromatic action spectra can provide indications on the effects of UV-B without interference due to processes induced by other radiation bands and are very useful to demonstrate a direct photochemical effect on a specific target.

However, studies conducted using monochromatic irradiation do not allow for photoregulated compensatory mechanisms which occur in nature, such as photorepair and the induction of protective UV-absorbing compounds. A complementary approach, which takes into account information from both monochromatic and polychromatic irradiation conditions, seems to be most adequate to describe the complex biological responses to UV of whole, intact organisms.

Suggested reading

Holmes, M.G., Action spectra for UV-B effects on plants: monochromatic and polychromatic approaches for analysing plant responses, in *Plant and UV-B: responses to environmental change*, Cambridge University Press, Lumsden, P.J. (Ed.), Cambridge, 1997, 31.

7. Photosensory biology (Lenci)

Friday morning, basic symposium (Lenci)

Besides being a fundamental source of energy for all photosynthetic organisms and microorganisms, light is an environmental stimulus of primary importance for all living beings, terrestrial and aquatic, diurnal and nocturnal, prey and predator, alike. The importance of light as a stimulus applies for creatures provided with "eyes" and neural networks as well as for aneural life forms like plants, fungi and even unicellular microorganisms, such as bacteria, algae and protozoa. In all living organisms movement is one of the most important outcomes of the complex interaction and communication of an organism with the environment. The lectures will be focused on photomovements of microorganisms.

As a matter of fact, many freely motile microorganisms are provided with a photoreceptor apparatus able to perceive the quantity and the quality of light in the environment and to transform the absorption of a photon into a biophysical or biochemical signal which can be recognized, elaborated and transduced by the living system. The main photobehavioral responses and the experimental techniques used to study them will be described.

The multi- inter-disciplinary approaches to investigate the mechanism by which the photoreceptors transmit information about the characteristics of a light signal to the downstream transduction pathway components will be discussed.

Saturday after lunch, special symposium (Columbetti)

Photomovements in microorganisms

Photomovements can be defined as alteration in cells movements caused by light. As already mentioned in a previous lecture, they belong to the larger field of photosensory biology. Though the study of photomovements is out of fashion in these days, it may be worthwhile trying to understand why it is important and why generations of scientists have dedicated to it long hours at the microscopes in the early times and then have developed more and more complicated measurement techniques, such as the modern automated tracking systems. The main reason for this is that to measure photomovements is exactly equivalent to asking a man whether he sees a red or a yellow light. In other words, to perform a measurement of phototaxis simply means to ask the cell a question and record the answer, where the question is the light stimulus and the answer the change in movement. In fact, the question can get a bit more complicated, and one can "ask" using different fluence rates, different wavelengths and even different polarizations. It's a sort of

psychophysics experiment, where, instead of recording simple answers to questions or measuring sophisticated electrophysiological responses, the researcher simply studies a behavior. The major difference is that in the case of higher systems the behavior may be complex, while in the case of microorganisms it is usually stereotyped. Of course, this approach is not exhaustive and it cannot give in itself a definite answer as to the nature of the receptor pigments or to the steps of the subsequent dark chain of molecular events that take place in a cell after light absorption.

Notwithstanding these apparent limitations, the study of photomovement has been for years, and is even now, a very useful and relatively simple tool to start getting information on the cell machinery underlying photosensory responses in microorganism. After all, one should not forget that the first steps in the investigation of the sophisticated mechanisms of chemotaxis started with the measurement of ring formations in simple Petri dishes. Furthermore, the behavioral approach can be used to test the predictions of more sophisticated molecular models. Photobehavior can be studied at the level of single cells or of cells populations. In both cases the modern techniques of image analysis coupled to CCD cameras have made it possible to significantly improve the precision of measurements and to make more reliable models that have helped clarify some aspects of, for instance, oriented movement of cells with respect to light direction.

8. Photomedicine

Saturday morning, basic symposium (Trautinger and Miranda)

Basic photodermatology (Trautinger)

Abstract: Photodermatology deals with the clinical consequences of the interaction of ultraviolet radiation with the various molecular and cellular components of human skin. These responses can be either physiologic (e.g. Vitamin D production, tanning) or lead to adverse reactions and disease (e.g. sunburn, polymorphic light reaction, and other photosensitivity diseases). Furthermore, sunlight and ultraviolet radiation from specific artificial sources (UVB, narrow-band UVB, UVA1) alone or in combination with photosensitizing drugs (psoralen-photochemotherapy) can be therapeutically employed for the treatment of a wide range of skin and other diseases. Finally, photodermatology also includes photoprotection through avoidance, clothing, and sunscreens with the main aim to prevent photoaging and photocarcinogenesis.

The lecture will provide basic knowledge about these major areas in photodermatology.

Photoreactivity and phototoxicity of drugs (Miranda)

- Photoreactivity of drugs under sunlight: photostability and its implications.
- Drugs as photosensitizers: desired and undesired effects.
- Major types of photosensitizing drugs.
- Predicting and understanding the phototoxicity of drugs: screening tests and mechanistic assays.
- Photophysics of drugs. Fluorescence measurements and laser flash photolysis studies.
- Photochemistry of drugs. Identification of photoproducts and elucidation of the involved photochemical mechanisms.
- Interaction of drug excited states with biomolecules.
- Photosensitization by drug metabolites and photoproducts.
- Photochemistry of sunscreens: photoprotection, photostability and photosensitization.

Saturday after lunch, special symposium (Trautinger and Miranda)

Phototherapy: Specific treatment modalities (Trautinger)

The lecture will provide an overview of the various currently used methods of phototherapy and psoralen photochemotherapy. Treatment of psoriasis, atopic dermatitis, cutaneous T-cell lymphomas, vitiligo and other skin diseases will be covered. Special emphasis will be given to extracorporeal photochemotherapy (photopheresis). Methods of radiation delivery, mechanisms of action, clinical results, and adverse reactions will be discussed.

Photooxidative reactions of drugs with biomolecules (Miranda)

- Oxidative damage photosensitized by drugs. Type I and type II mechanisms.
- Drug-mediated photooxidation of lipids. Polyunsaturated fatty acids and cholesterol.

- Photoreactivity of drugs with proteins. Oxidation of amino acid residues.
- Covalent photobinding of drug to proteins. Photoantigen formation and its implications in photoallergy.
- Photooxidative DNA damage mediated by drugs. Reactions at the purine bases and at the deoxyribose units.
- Drugs as triplet sensitizers for the photodimerization of pyrimidine bases in DNA.
- Repair of damaged DNA by photoinduced electron transfer. Drugs with potential photolyase activity.