PL101

Vitamin D and photoprotection: progress to date

Katie Marie Dixon

Department of Anatomy and Histology, Bosch Institute, University of Sydney, Sydney, NSW, Australia

Vitamin D compounds are produced in the skin following exposure to ultraviolet radiation (UVR). We previously reported that vitamin D compounds protect against UVR-induced cell death and DNA damage (cyclobutane pyrimidine dimers; CPD and 8-oxoguanine) in cultured human skin cells. We have further evidence that these photoprotective effects are mediated by the non-genomic pathway for vitamin D. We used an in vivo model to investigate the photoprotective effects of topical 1,25-dihydroxyvitamin D3 (1,25D) and low calcemic analogs (including a non-genomic agonist) in Skh:hr-1 mice exposed to 3 MED of UVR, showing significant reductions in apoptotic sunburn cells (SBCs) and CPDs. Topical 1,25D and analogs also significantly reduced UVR-induced immunosuppression in these mice. Moreover, topical application of 1,25D or low calcemic non-genomic agonist JN proved to be protective in a photocarcinogenesis model in which mice were exposed to chronic low dose UVR. The average number of tumours per tumour-bearing mouse was reduced from 5.0±0.8 in vehicle treated mice to 1.7±0.3 ($p < 0.01$) and 2.9±0.4 ($p < 0.05$) in 1,25D and JN treated mice respectively. The latency to tumour onset was significantly greater in 1,25D treated mice compared with vehicle treated mice ($p < 0.05$). At the conclusion of the 40 week study, squamous cell carcinoma incidence was reduced from 42% to 17% and 33% in 1,25D and JN groups respectively. In separate human studies, biopsies from subjects treated topically with 1,25D (240pmol/cm^2) 24h prior to and immediately after UVR (2 MED) showed reductions in SBCs measured 24h after UVR from 4.5±1.7 per mm to 1.4±1.5 ($p<0.05$). CPD were reduced from 12.0±2.5% to 1.0±0.2% ($p < 0.001$) in subjects treated with 1,25D. We recently showed similar reductions in CPD and also 8-oxoguanine when 1,25D was applied to human subjects immediately after UVR only. The effects of topical 1,25D on UVR-induced suppression of immune response to a recall antigen were examined using the Mantoux model in humans. Although higher doses of topical 1,25D were immunosuppressive, topical 1,25D (24 pmol/cm^2) was not immunosuppressive alone but had no effect on UVR-induced suppression of erythema to this recall antigen. These studies indicate that 1,25D is photoprotective for a variety of endpoints in humans, mice and cultured cells, and resulted in the first comprehensive report of a protective role for vitamin D compounds in UVR-induced skin carcinogenesis.

IL102

Response to RBAc-induced photodamage to HeLa cells: only cell death induction?

Luciana Dini

Department of Biological and Environmental Sciences and Technologies (Di.S.Te.B.A.) University of Salento, Lecce, Italy

Resistance to cell death and overruling immunosurveillance represent two fateful hallmarks acquired by tumour cells. Thus, the ideal cancer treatment should merge the direct cytotoxic action on tumour cells with potent immunostimulatory effects. In this context, PhotoDynamic Therapy (PDT) is a promising cancer treatment for its efficiency in i) cell death induction; ii) high selectivity for tumour cells; iii) ignition of the immunogenic cell death; iiii) triggering immune response upon dead cells removal. Cancer PDT is based on the cellular oxidative damage following ROS generation upon visible light activation of a cell-localized PhotoSensitizer (PS). The interaction between light, cell or tissue molecular oxygen and PS gets the photodynamic reaction. The PS subcellular localization dictates the primary site of damage and the consequent outcome of the treatment, implying direct cell damage (i.e., cytotoxicity eliciting apoptotic and/or autophagic and/or necrotic cell death) and secondary effects (i.e., damage to the vasculature and inflammatory reaction ending in the systemic immunity). In recent times, more and more efforts are addressed to recognize the better PS employing in cancer PDT. Rose Bengal Acetate (RBAc), administrated for 60 minutes at 10^{-5}M and activated with 1,6 J/cm^2 green light, is a powerful PS. It is able to initiate in HeLa cells several signaling processes leading to rapid, independent, successive, long-lasting and time-related onset of apoptosis and autophagic cell death by signals originating from or converging on almost all intracellular organelles, i.e. mitochondria, lysosomes, Golgi apparatus and ER, despite RBAc primary perinuclear localization. Particularly, apoptosis occurs as early as 1h after PDT *via* activation of intrinsic pathway, followed by activation of extrinsic, caspase-12-dependent and caspase-independent pathways. The clearance of RBAc photokilled HeLa cells, in terms of recruitment, recognition and removal, is very efficient both *in vitro* and *in vivo*. HeLa cells recruit phagocytes through release of fractalkine, and are recognized by changes in cell surface amount and distribution of phosphatidylserine and glycans. Finally, macrophages internalize RBAc-treated HeLa cells and release IL-10, TGF-β and TNF-α that along with translocation and secretion of HSP70 by dead cells suggest that RBAc-PDT can elicit a positive immunomodulatory response. In conclusion, RBAc-induced photodamage commits not only a potent cell death induction but also influences immune system to counteract cancer cells.

OC103

In vivo PDT with a novel sulfonamide bacteriochlorin: treatment optimization and the role of the immune system

Luís B. Rocha[1], Janusz M. Dąbrowski[2], Luís G. Arnaut[1], Mariette Pereira[3], Sérgio Simões[4]

[1]Luzitin, SA, Coimbra, Portugal; [2]Jagiellonian University, Kraków, Poland; [3]Chemistry Department - University of Coimbra, Coimbra, Portugal; [4]Bluepharma - Indústria Farmacêutica SA, Coimbra, Portugal

Photodynamic Therapy (PDT) is a non-invasive, safe and clinically-approved procedure that in the recent years is increasingly been recognized as a promising anticancer therapeutic strategy. The PDT reaction produces reactive oxygen species that are directly responsible for tumour cell and tumour vasculature destruction. It is now widely accepted that some PDT protocols can also induce a systemic and tumour-specific immune response, which can contribute to the elimination of the primary tumour and also to the destruction of distant metastasis.

We are currently developing a novel sulfonamide bacteriochlorin (LUZ11) with properties approaching those of an ideal PDT photosensitizer: simple and affordable synthesis, high purity and stability, molar absorptivity of 137,000 $M^{-1}cm^{-1}$ at 744 nm, high yields of singlet oxygen and hydroxyl radical formation, and solubility in biocompatible formulations. After *in vitro* drug screening studies, a formulation for IV administration of LUZ11 was developed and optimized. The preliminary safety pharmacology studies revealed no signs of toxic reactions. The proof-of-concept was demonstrated in a mouse tumour model in which complete and long term tumour destruction was obtained in one single vascular-PDT session.

We now report the optimization of a vascular-PDT protocol. We show that 1 mg/kg of LUZ11, 15 minutes drug-to-light interval and 6.5 minutes laser irradiation at 173 mW (67 J) is 100% safe and cures 87.5% of the mice ("cure" is defined as the absence of palpable tumor 60 days after the treatment). A preliminary evaluation of the antitumor immune response induced by LUZ11 vascular-PDT showed a significant increase (p=0.03) in the median survival of mice with bilateral tumours (1 in each leg), in which only one tumour was treated (when compared to non-treated control). This seems to indicate that PDT can have a systemic effect against non-treated tumors. Moreover, we show that 38% of the mice previously cured with LUZ11 vascular-PDT

were able to rejected a rechallenge with the same tumour cells (> 3 months after PDT treatment), while the control group with surgically removed tumors were unable to reject the rechallange. This suggests that PDT is able to induce a sustained antitumor immune memory.

IL104

Pro-Survival Signaling Associated with Stress-Induced Nitric Oxide in Photodynamically Challenged Tumor Cells

Albert W Girotti, Reshma Bhowmick

Medical College of Wisconsin, Milwaukee, WI, United States

Many tumors exploit nitric oxide (NO) as an anti-apoptotic/pro-survival effector molecule. We recently discovered that two human breast cancer cell lines rapidly upregulated inducible nitric oxide synthase (iNOS) after being subjected to photodynamic stress sensitized by 5-aminolevulinic acid (ALA)-generated protoporphyrin IX in mitochondria. Apoptotic cell photokilling was strongly enhanced by iNOS inhibition, iNOS knockdown, or NO scavenging, indicating that iNOS/NO was acting cytoprotectively. Key signaling events associated with this response have been identified in human breast cancer COH-BR1 cells. Immunocytochemistry and Western analysis revealed a cytosol-to-nucleus translocation of transcription factor NF-κB in photostressed cells. Bay 11-7082, an NF-κB activation inhibitor, suppressed translocation and iNOS induction while stimulating apoptosis. This suggests that NF-κB played a key role in iNOS transcriptional activation. Wortmannin, a PI3K inhibitor, stimulated photostress-induced apoptosis while suppressing Akt phosphorylation, NF-κB activation, and iNOS induction, thus implicating Akt activation in the latter. In other aspects of cytoprotective signaling, iNOS inhibition or knockdown revealed that activation of pro-apoptotic JNK and p38 MAPK was suppressed by NO, possibly via S-nitrosation. However, possible activation of protein kinase GbycGMP from NO-stimulated guanylylcyclase was ruled out. We also showed that iNOS-knockdown increased p53 expression under photostress but negated surviving induction, consistent with pro-apoptotic p53 being negatively regulated by NO and anti-apoptotic survivin being positively regulated. More recent studies revealed that ALA/light-stressed prostate cancer PC-3 cells also upregulate iNOS and NO as a cytoprotective strategy. Moreover, cells surviving photostress exhibited a striking NO-dependent growth spurt with a large increase in cell cycle S-phase occupancy. Our studies suggest that iNOS/NO induction may be a common response of tumor cells to PDT stress. If so, this could have a serious negative impact on treatment efficacy unless dealt with by rational interventions, e.g. use of appropriate iNOS inhibitors.

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OC105

5-ALA-PDT induces RIP3-dependent necrosis in glioblastoma.

Isabelle Coupienne, Grégory Fettweis, Jacques Piette

Virology and Immunology, GIGA-ST, University of Liège, Liège, Belgium

Glioblastoma constitute the most frequent and deadliest brain tumors of astrocytic origin. They are resistant to all current therapies and are associated with a high rate of recurrence. Glioblastoma were previously shown to mainly respond to treatments by 5-aminolevulinic acid (5-ALA)-based photodynamic therapy (PDT) by activating a necrotic type of cell death. The receptor-interacting protein 3 (RIP3) has recently been outlined as a key mediator of this caspase-independent form of programmed cell death.

In the present study, we analyzed the necrotic mechanism induced by 5-ALA-PDT in human glioblastoma cells and explored the role of RIP3 in this context. Our results show that PDT-induced necrosis is dependent on RIP3, which forms aggregates and colocalizes with RIP1 following photosensitization. We demonstrate that PDT-mediated singlet oxygen production is the cause of RIP3-dependent necrotic pathway activation. We also prove that PDT induces the formation of a pro-necrotic complex containing RIP3 and RIP1 but lacking caspase-8 and FADD, two proteins usually part of the necrosome when TNF-α is used as a stimulus. Thus, we hypothesize that PDT might lead to the formation of a different necrosome whose components, besides RIP1 and RIP3, are still unknown. The composition of the PDT-induced pro-necrotic complex is currently under investigation in the lab.

Furthermore, in most cases, glioblastoma are characterized by a constitutive activation of NF-κB. This factor is a key regulator of various processes such as inflammation, immune response, cell growth or apoptosis. Its inhibition was shown to further sensitize glioblastoma cells to PDT-induced necrosis, however, no difference in RIP3 upshift or aggregation could be observed when NF-κB was inhibited.

IL106

Optimizing Vascular Responses to Photodynamic Therapy

Theresa Busch

University of Pennsylvania, Philadelphia, PA, United States

The tumor microenvironment, including the structure and function of its vasculature, is an effector of tumor response to many therapeutic interventions. Photodynamic therapy (PDT) is not exempt from the limitations imposed by tumor microenvironment. Tumor vascularization can play a role in photosensitizer and oxygen delivery. PDT damage of tumor-supporting blood vessels, which contributes to tumor cell death through nutrient/oxygen deprivation, may also trigger the activation of protective responses. We have investigated the mechanisms by which the structure of tumor blood vessels and signaling by their component cells can alter tumor response to PDT. We seek to develop methods of priming tumor vasculature to insult by PDT, and herein focus on studies of two pharmaceutical-mediated and clinically-relevant approaches for sensitization of tumor blood vessels to PDT. Studies were conducted in murine fibrosarcoma (RIF) or one of several human tumor xenografts (e.g., H460 non-small cell lung carcinoma). To prime tumor vasculature to PDT, animals were administered either an antibody against vascular endothelial growth factor (VEGF) or a small molecule inhibitor of epidermal growth factor receptor (EGFR) for two days prior to light delivery. Mice treated with anti-VEGF antibody as a priming agent exhibited an altered vascular phenotype in their tumors, demonstrated greater decreases in tumor perfusion after PDT, and experienced better long-term outcomes. Inhibition of EGFR in the time frame before PDT led to increases in PDT-mediated vascular damage, along with direct effects on tumor cells and large increases in curative outcome. Together these data suggest that altering the structural composition of the tumor vasculature and/or survival signaling by its endothelium can augment vascular response to PDT. We conclude that pre-PDT priming of tumor vasculature can be used to improve responsiveness to therapy.

OC107

Lymphatic-specific photodynamic therapy in the mouse dermis

Witold W Kilarski[1], Angelika Muchowicz[2], Malgorzata Wachowska[2], Renata Mężyk-Kopeć[3], Jakub Golab[2], Melody A. Swartz[1], Patrycja Nowak-Sliwinska[1]

[1]Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland; [2]Medical University of Warsaw, Warsaw, Poland; [3]Jagiellonian University, Krakow, Poland

Lymphatic vessels transport fluid, antigens and immune cells to the draining lymph nodes to orchestrate adaptive immune responses. Lymphatic vessels have been associated with inflammation, cancer metastasis, autoimmunity, tolerance and

transplant rejection, and thus, blocking lymphatic transport is a potential therapeutic strategy for treating such pathologies. Here we define conditions that lead to specific and local closure of the lymphatic vasculature using photodynamic therapy (PDT). Lymphatic-specific PDT was performed by intradermal administration of the photosensitizer verteporfin that effectively accumulates within lymphatic vessels. At certain light fluencies and photosensitizer concentrations, activation of verteporfin accumulated within collectors led to production of reactive oxygen species selectively within the lymphatic vessels and induced necrosis of their endothelial cells and pericytes, leaving basement membrane intact. Depending on the verteporfin dose, lymphatic-PDT functionally blocked collecting lymphatics or both, lymphatics and blood vessels. Regeneration time was light fluence-dependent and the 'ghost lymphatic' vessels had regenerated their endothelial lining and became re-perfused, with a characteristic hyperplasia of peri-lymphatic smooth muscle cells. Restoration of lymphatic function was found to be independent of neovascular growth, but rather governed by the epimorphic-like, lymphangiogenesis-independent regeneration.

OC108

The mechanism of pharmacological restoration of TAp73 tumor suppressor function by protoporphyrin IX

Joanna Zawacka-Pankau

Karolinska Institutet, Stockholm, Sweden

Protoporphyrin IX (PpIX), is the only photosensitizer produced endogenously upon administration of pro-drug, aminolevulinic acid. We have shown before that PpIX *per se*, can induce apoptosis in cancer cells prior to light illumination (1). In the follow-up study we revealed that PpIX targets p53 *in vitro* and induces p53-dependent and independent cell death using a pair of isogenic colon cancer cells HCT 116 differing only in p53 status (2). Furthermore, cancer cells expressing wild-type p53 were more sensitive to photodynamic reaction than p53-null cells. Due to high structural and functional homology between p53 family members, we reasoned that p53 null cancer cells undergo apoptosis upon PpIX treatment due to pharmacologically activated p73. We have already shown that PpIX binds p73 transactivation domain. Our recent findings reveal that in dark, PpIX induces p73 levels which promotes PUMA and NOXA expression and consequent apoptosis of cancer cells. We have also pinned down the mechanism of p73 restoration in cancer cells upon PpIX converging on the release of p73 from inhibitory complex with MDM" protein.

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IL109

Unfolding the role of ER stress in photodynamic therapy

Patrizia Agostinis, Tom Verfaillie, Noemi Rubio Romero, Alex Van Vliet

University of Leuven, Faculty of Medicine, Department of Molecular & Cell Biology, Leuven, Belgium

Endoplasmic reticulum (ER) stress is emerging as an important modulator of different pathologies and as a mechanism contributing to cancer cell death in response to therapeutic agents. In several instances oxidative stress and the onset of ER stress occur together, yet the molecular events linking reactive oxygen species (ROS) to ER stress-mediated apoptosis are still elusive. We used hypericin, a photosensitizer accumulating prevalently to the ER, to investigate the molecular and signaling mechanisms underlying ROS-induced ER stress and cell death. We report that Hyp-PDT activates the major axis of the unfolded protein response and causes CHOP- and Noxa-mediated apoptotic cell death. We also discovered that the Ser/Thr kinase PERK, a key ER-stress sensor of the UPR, is uniquely enriched at the mitochondria-associated ER membranes (MAMs). $PERK^{-/-}$ cells display disturbed ER morphology and Ca^{2+} signaling as well as significantly weaker ER-mitochondria contact sites. Re-expression of a kinase dead-PERK mutant but not the cytoplasmic deletion mutant of PERK in the $PERK^{-/-}$ cells, re-establishes ER-mitochondria juxtapositions and mitochondrial sensitization to hypericin-mediated PDT, indicating that although PERK-kinase activity is dispensable for preserving the ER-mitochondria interaction its cytoplasmic domain is required. During PDT-mediated ER stress PERK contributes to apoptosis twofold; by sustaining the levels of pro-apoptotic CHOP and by facilitating the propagation of ROS signals between the ER and mitochondria through its tethering function.

IL110

Mechanisms of photo-oxidative damage to cellular antioxidant systems

Michael J. Davies

The Heart Research Institute, Sydney, Australia

Mammalian tissues contain >25 selenoproteins that incorporate selenocysteine (Sec) or selenomethionine (SeMet) residues. These include key antioxidant enzymes including glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). Both Sec and SeMet residues are readily oxidized due to their low oxidation potentials, and (for Sec) a low pKa, which results in this residue being present predominantly in its ionized form (pKa ~5.5, cf. 8.4 for Cys). These species may therefore be major targets for oxidants.

In this study, the oxidative damage to GPx and TrxR by a Rose Bengal / visible light system, which generates singlet oxygen ($^1O_2$) was assessed. Exposure of GPx or TrxR, either as purified proteins or in cell lysates, to Rose Bengal and visible light resulted in significant, time-dependent reductions in enzyme activity (10–40%, $p < 0.05$). $^1O_2$ appears to be a major source of damage, as greater inhibition (ca. 2-fold) was detected in D_2O. No additional inhibition was detected on prolonged incubation post-photolysis, eliminating a role for photo-products. Methionine, which is rapidly oxidised by $^1O_2$ ($k \sim 10^7$ M^{-1} s^{-1}) reduced the extent of photo-inactivation when high molar excesses were present indicating that reaction of $^1O_2$ with Sec residues is very rapid; this has been confirmed in direct kinetic studies of $^1O_2$ with Sec and SeMet. Reductants ($NaBH_4$, dithiothreitol, GSH, and NADPH) added after the cessation of photolysis were unable to reverse this damage. Evidence has been obtained (non-reducible) aggregates with both GPx and TrxR, but limited fragmentation. Protein carbonyls were also detected on TrxR, but not GPx.

Additonal studies have examined the ability of SeMet (and its sulphur analogue, Met) to scavenge photo-generated peroxides both directly, and catalytically in the presence of glutathione (GSH) or thioredoxin reductase (TR)–thioredoxin (Trx) systems. SeMet, but not Met, reacts rapidly with H_2O_2, peptide-/protein-peroxides, and plasma peroxides. Such reactions generate the selenoxide (from SeMet) and sulphoxide (from Met). In the presence GSH or a TR – Trx reducing system, redox cycling between the selenoxide and reduced SeMet is observed, with this enhancing peroxide removal; this does not occur with Met under these conditions. Overall, these data indicate that seleno residues can be major targets for oxidation and play an important role in cellular defences against photo-oxidative damage, with low-molecular-mass species such as SeMet being able to act as a *catalytic* protective agent.

IL111

^{18}O-Labeled peroxides and singlet molecular oxygen as valuable tools for studying the role of $^1O_2$ in biological system

Paolo Di Mascio

Universidade de São Paulo, Instituto de Química, Departamento de Bioquímica, São Paulo, Brazil

In mammalian tissues, ultraweak chemiluminescence arising from biomolecules oxidation has been attributed by several authors to the radiative deactivation of singlet molecular oxygen [$O_2(^1\Delta_g)$] and excited triplet carbonyl products as dioxetane intermediates. Singlet dioxygen has been shown to be generated in biological systems and have been implicated in cell defense mechanisms and dark reaction. The decomposition of some organic hydroperoxides into peroxyl radicals is known to be a potential source of $O_2(^1\Delta_g)$ in biological systems. As examples, we will discuss the generation of $O_2(^1\Delta_g)$ from lipid hydroperoxides (LOOH), which involves a cyclic mechanism from a linear tetraoxide intermediate originally proposed by Russell. In addition to LOOH, other biological LOOH, may also participate in reactions leading to the generation of $O_2(^1\Delta_g)$. The approach used to unequivocally demonstrate the generation of $O_2(^1\Delta_g)$ in these reactions is the use of ^{18}O-labeled hydroperoxide/triplet dioxygen ($^{18}[O_2(^3\Sigma^-_g)]$), the detection of labeled compounds by HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) and the direct spectroscopic detection and characterization of $O_2(^1\Delta_g)$ light emission. Characteristic light emission at 1,270 nm, corresponding to the singlet delta state monomolecular decay was observed. Moreover, the presence of $O_2(^1\Delta_g)$ was unequivocally demonstrated using the direct spectral characterization of near-infrared light emission. Using $^{18}[O_2(^3\Sigma^-_g)]$, we observed the formation of ^{18}O-labeled $O_2(^1\Delta_g)$ ($^{18}[O_2(^1\Delta_g)]$) by the chemical trapping of $^{18}O_2(^1\Delta_g)$ with the anthracene-9,10-diyldiethane-2,1-diyl disulfate disodium salt (EAS) and detected the corresponding ^{18}O-labeled EAS endoperoxide using HPLC-MS/MS. To investigate the effect of $O_2(^1\Delta_g)$ on biomolecules such as DNA, we have devoted efforts to develop suitable $O_2(^1\Delta_g)$ generators. The combined use of the thermolysis of a water-soluble naphthalene endoperoxide as a generator of $^{18}[O_2(^1\Delta_g)]$ and the sensitivity of HPLC-MS/MS allowed the study of $O_2(^1\Delta_g)$ reactivity toward biomolecules. Photoemission properties and chemical trapping clearly demonstrate that the production of hydroperoxide and excited carbonyls generates $^{18}O_2(^1\Delta_g)$, and points to the involvement of $O_2(^1\Delta_g)$ in physiological and pathophysiological mechanisms.

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IL112

Interaction of UV radiation with DNA: excited states and reactivity

Dimitra Markovitsi

CNRS, Gif-sur-Yvette, France

The talk will focus on processes taking place when UV photons are absorbed directly by DNA. Experimental results will be discussed in the light of recent theoretical developments.

Due to electronic coupling, the excited states responsible for photon absorption may extend over two or more bases. Thus, in contrast to isolated bases, UVA radiation is weakly absorbed by single and double strands resulting to formation of cyclobutane thymine dimers; base-pairing enhances photon absorption and favours thymine dimerization.

Delocalized excited states lead to ultrafast energy transfer among the bases of duplexes and guanine quadruplexes. In the case of calf thymus DNA, the energy transfer process continues on the nanosecond time-scale, possibly mediated by a charge transfer process.

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IL113

Photosensitized triplet-triplet energy transfer in DNA: formation of cytosine-containing cyclobutane dimers

Thierry Douki

INAC/SCIB/LAN, J. Fourier University & CEA-Grenoble, Grenoble, France

Cyclobutane pyrimidine dimers (CPDs) are major DNA lesions responsible for the mutagenic properties of UV radiation. These photoproducts are induced either by direct absorption of UV photons by DNA bases or by a photosensitized reaction known as triplet-triplet energy transfer (TTET). TTET is mediated by a wide range of molecules, including drugs, in the presence of UVA. Because thymine exhibits the lowest energy for its triplet excited state, TTET is mostly discussed in terms of TT (thymine-thymine) CPD.

In the present study, we determined the complete distribution of CPDs which includes TT, TC (thymine-cytosine), CT (cytosine-thymine) and CC (cytosine-cytosine) derivatives. In aqueous solution of calf thymus DNA, TTET mediated by fluoroquinolones and aromatic ketones leads to the overwhelming formation of TT CPD. TC and CT CPDs are present in much lower amounts. The study was then extended to two other genomic DNAs of bacterial origin exhibiting either a high or a low proportion of TT dinucleotide sequences. A nice correlation was found between the TT content of DNA and the yield of CPDs. Yet, in the three types of DNA, the proportion of TT CPD with respect to TC and CT CPDs is much larger than expected from a simple statistical prediction. In addition, CC CPDs were unambiguously detected although in small amounts.

This set of data suggests that the classical mechanism of TTET where the triplet energy of the excited sensitizer is transferred to a thymine has to be partly revisited. The role of the adjacent pyrimidine base cannot be neglected. Recent results on the delocalization of the excited states in DNA could be related to our observations. This idea of a major role of the duplex structure is confirmed by the observation of the formation of CC CPD. The triplet energy of cytosine is larger than that of the photosensitizers used and no TTET should take place.

OC114

Relevancy of a double efficacy against glycation and UVA to protect human skin from photoaging

Louis Danoux, Florence Henry, Solene Mine, Christine Jeanmaire, Olga Freis, Alain Denis

BASF BEAUTY CARE SOLUTIONS FRANCE SAS, Essey-lès-Nancy, France

As well as UV irradiation, glycation is now recognized as a causative factor of skin aging. Glycation is a complex process initiated by condensation of reducing sugars and primary amine groups of proteins into Schiff's bases. Then Schiff's bases undergo a slow rearrangement which ends in the formation of various and stable Advanced Glycation End-products (AGEs) such as Carboxy-Methyl-Lysine and Pentosidine. AGEs accumulate mainly on macromolecules of dermal matrix having a slow turn-over such as collagen and elastin and cause the formation of cross-links between proteins, which leads to the skin yellowing and stiffening with a loss of skin elasticity. During its progress, glycation can generate an oxidative stress which shares many characteristics with UVA irradiation. Glycation as well as UVA promote the release of Reactive Oxygen Species (ROS), Reactive Carbonyl Species (RCS) and activation of transition metals which globally results in cell toxicity and inflammation. Especially in human skin, UVA and Glycation synergize each other because glycated proteins enhance the UVA-induced oxidative stress, and reciprocally UVA-induced ROS and RCS worsen the glycation process. Therefore; a double efficacy against glycation and UVA seems very relevant for ingredients dedicated to the protection of human skin from photoaging.

With this aim, a Plant Extract (PE) rich in antioxidant molecules was selected and its potential to inhibit glycation and UVA toxicity was evaluated. The inhibition of glycation was established in tubo on key proteins of skin: albumin and elastin. The effect on UVA was evaluated on human fibroblasts. The glycation-related oxidative stress was addressed by the assays of copper-induced Low Density Lipoproteins oxidation and interleukine 8 release from glyoxal treated fibroblasts. To study the interaction between glycation and UVA, an original model based on skin biopsies was developed. Through this *ex-vivo* model, we demonstrated by immunohistochemistry that UVA enhances the glycation of the dermis and then by the use of Electron Spin Resonance, that glycation potentiates the UVA-mediated release of free radicals (FR). PE revealed an interesting inhibition of FR release from UVA-irradiated skin biopsies. The anti-oxidative potential of PE was confirmed by 2 clinical assays (30+/-2 years old and 66+/-1 years old) based on measurement of UV-induced chemiluminescence of human skin. To conclude, the PE represented an attractive way to reduce apparent skin photoaging by interfering with pathways synergistically promoted by glycation and UVA.

OC115

Vitamin-D3 photosynthesis: from action-spectrum to action-matrix

Arjan van Dijk, Henk van Kranen, Harry Slaper

RIVM, Bilthoven, Netherlands

In 2009, Norval, Björn and De Gruijl raised the question "Is the action spectrum for the UV-induced production of previtamin D3 in human skin correct?" We have taken the challenge and made an attempt to derive relations for the spectral dependence of all photochemically relevant processes in the synthesis of vitamin-D. We started out with the construction of a chemical reaction set for all conversions that include pro-vitamin-D, pre-vitamin-D, lumisterol, tachysterol and cholecalciferol. Suprasterols and trans-vitamins were left our due to lack of supporting experimental data. From literature, we collected measured spectra for (skin-)transmission, absorbances and quantum yields. These measured spectra form the basis for our estimate of a whole action-spectrum matrix, as opposed to the classical single CIE-action-spectrum that is still based on MacLaughlins work from 1982. Then, we used the resulting action-matrix to simulate the experiment performed in June 1986 by Webb, Kline and Holick, who followed the chemical conversions in a quartz tank on their roof in Boston, starting with just pro-vitamin-D. Remote-sensing estimates for ozone and cloud-reflection from NIMBUS were used in combination with a TUV-derived atmospheric radiative transfer model to construct a time-series for the irradiance that must have been available during the experiment. Despite the substance-specific spectral detail of our simulation, the correlation of our modeled concentrations with the experiment was rather poor. One explanation for the mis-match may be that our set of reactions is incomplete. A different explanation can be that the experimentally estimated values for quantum yields and absorbances, that we used in our derivation of the action-matrix, are highly uncertain or only valid under different conditions. Our failure to simulate a simple in-vitro experiment prevented us from using the action-matrix concept to simulate the photo-synthesis of vitamin-D in real skin. In this presentation we will show our derivation of the action-matrix, demonstrate the simulation of the Boston experiment and discuss options for improvement of the model.

OC116

Fibrillin microfibril mass is reduced in photoaged skin

Elizabeth C Naylor, Rachel E B Watson, Christopher E M Griffiths, Michael J Sherratt

University of Manchester, Manchester, United Kingdom

Photoageing of human skin is caused by chronic exposure to ultraviolet radiation (UVR) and is characterised, in part, by extensive remodelling of the dermal elastic fibre network. Key components of this network, fibrillin microfibrils, are complex beaded macromolecular assemblies that are rich in UVR-absorbing amino acid residues (Cys, His, Phe, Trp and Tyr). Although microfibril mass is thought to be invariant in young healthy adult tissues we have previously demonstrated a dose-dependent loss of mass from microfibrils irradiated by broadband UVB *in vitro*. Here, we hypothesise that microfibril mass will also be reduced by chronic exposure to UVR *in vivo*.

Healthy, but severely photoaged, volunteers were recruited to the study (n=5; 3M, 2F; age range: 66-80 years). Samples (3mm skin biopsies) were obtained from photoprotected upper inner arm and photoaged extensor forearm. Microfibrils were extracted using bacterial collagenase and size exclusion chromatography prior to imaging via scanning transmission electron microscopy (STEM) in order to characterise their mass distribution (n=5 individuals, n= 250 repeats per extracted sample).

The mass per repeat of microfibrils extracted from photoprotected skin (2577kDa, SEM 65kDa) was similar to that previously reported for adult tissues (~2600kDa). In contrast the mass per repeat of microfibrils extracted from photoaged skin (2290kDa, SEM 47kDa) was significantly reduced (paired Student's T-test, $p = 0.039$).

These data demonstrate that chronic photoageing *in vivo* results in a significant remodelling of fibrillin microfibril structure. However, it is not known whether these lower mass microfibrils result from either UVR denaturation and/or the synthesis of new assemblies. Future work will assess how these changes in molecular mass are related to the mechanical and biochemical functions of microfibrils resident in photoaged skin.

OC117

Oxidation of the human DNA repair protein XRCC3 by environmental factors: implication of intracellular glutathione

Pierre-Marie Girard[1], Dany Graindorge[1], Pascal Rigolet[1], Stefania Francesconi[1], Susan Scanlon[2], Evelyne Sage[1]

[1]Insitut Curie CNRS UMR3348, Orsay, France; [2]Yale university, New Haven, United States

In vertebrates, XRCC3 is one of the five Rad51 paralogs that plays a central role in homologous recombination (HR), a key pathway for maintaining genomic stability. While investigating on the overall harmful effects of UVA radiation, we discovered that photosensitization by UVA causes the oxidation of cysteine residues of human XRCC3 (hXRCC3) expressed in both human and chinese hamster ovarian (CHO) cells. hXRCC3 contents 8 cysteine residues and *in silico* prediction of its structure suggests that 6 of them (cys86, 141, 193, 221, 310 and 328) are potentially accessible to the solvent, with cys86 and cys328 spatially closed to each other. Using an antibody that recognises the C-terminal part of the protein, encompassing Cys328, we show by Western blots in non reducing conditions that hXRCC3 oxidation induced by photosensitization or by menadione (MN) leads to an increase in the electrophoretic mobility of the protein in CHO cells and to a decrease in the immunodetection of hXRCC3 in human cells. In both cell types, hXRCC3 oxidation is reversed by cellular reducing systems. The depletion of intracellular glutathione (GSH) by DL-buthionine-[*S,R*]-sulfoximine (BSO) does not prevent MN-induced cysteine oxidation of hXRCC3. Surprisingly, the need for GSH to trigger hXRCC3 oxidation after photosensitization by UVA will depend on the photosensitizer. Finally, mutation of all cysteine to serine residues of hXRCC3 leads to increase sensitivity of CHO cells to the DNA damaging agent camptothecin (CPT), highlighting a defect in HR, while mutation of cys86 or cys328 did not affect the cell sensitivity to CPT. Collectively, our results demonstrate that the DNA repair protein hXRCC3 is a new target of ROS induced by environmental factors and raise the possibility that the HR pathway could be regulated by the redox environment.

IL118

Guanine quadruplexes studied by femtosecond fluorescence spectroscopy

Pascale Changenet-Barret

CNRS, Gif sur Yvette, France

Guanines at millimolar concentration, *in vitro*, are well known to form peculiar quadruplex structures resulting from the hydrophobic stacking of several guanine quartets, in a planar arrangement. The interest in G-quadruplex structures has considerably grown after identification of various guanine-rich regions of the human genome able to form such architectures.[1] Despite the large number of publications devoted to G-quadruplexes, very few of them have addressed their intrinsic photophysics. Characterizing their optical properties and disentangling the various factors that may affect them are however prerequisites for the elucidation of the mechanisms underlying light-induced DNA damages.

In this respect, we undertook the study of the intrinsic photophysical properties of short G-quadruplexes formed upon the association of four single strands $d(TG_nT)$ in the presence of Na^+ or K^+.[2-5] Fluorescence decays were measured on a time scale spanning from 100 fs to 100 ns. We will show that when going from the monomeric chromophores to $d(TG_nT)_4$, both the absorption and fluorescence spectra change and the fluorescence decays become slower, revealing the importance of collective effects.[2,3] Our results suggest the existence of excited states delocalized over several bases, both for the Franck-Condon and the emitting state geometries. This delocalization is due to restricted conformational motions, specific for the G-quadruplexes, and is found to increase with the size of the quadruplex.[3,5] The higher mobility of Na^+ within the G-quadruplex structure, compared to K^+, is proposed to favor the trapping of the initially delocalized states by excited charge transfer states.[3]

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IL119

Molecular THz probes linked to biopolymers: direct mapping of hydration layer dynamics

Nikolaus P. Ernsting, Mohsen Sajadi

Dept. of Chemistry, Humboldt Universität zu Berlin, Berlin, Germany

The hydration layer dynamics of biopolymers can be obtained with fluorescent polarity probes, by recording their time-resolved Stokes shift. For this purpose, suitable probes were covalently linked to a disaccharide or into a DNA duplex, giving access to water molecules nearby. Also a new femtosecond fluorescence spectrograph was invented. Temperature dependent measurements prove that the probe truly reports the hydration dynamics. The local dielectric-loss spectrum epsilon”(omega) of the hydration layer is determined. For example, water around the disaccharide at room temperature has the same spectrum as free water at 4 °C: the sugar destructures the H-bonded network.

IL120

Photophysics of fluorescent proteins - dronpa and kaede

Stephen Meech

University of East Anglia, Norwich, United Kingdom

The growing family of fluorescent proteins exhibit a very wide array of photochemical phenomena, even though the chromophores and structures of the proteins are similar. We use ultrafast fluorescence up-conversion, transient absorption and transient IR to probe the origin of these differences. For example the photochromic protein dronpa has the same chromophore as GFP, but dramatically different photophysics. We probe the primary events in dronpa with time resolved IR. Similarly the ‘highlighter’ protein kaede exhibits a photochemical conversion never found in GFP itself. We synthesise the resulting chromophore and compare its photophysics with those of the GFP chromophore. The extended conjugation of kaede dramatically alters the photophysics.

OC121

Photochemistry of Flavin-Based Blue-Light Receptors Studied by Time-resolved Electron Paramagnetic Resonance

Till Biskup[1], Bernd Paulus[1], Kenichi Hitomi[2], Elizabeth D. Getzoff[2], Erik Schleicher[1], Stefan Weber[1]

[1]University of Freiburg, Institute of Physical Chemistry, Freiburg, Germany; [2]Scripps Research Institute, La Jolla, CA, United States

Photoactive blue-light sensitive proteins almost exclusively utilize flavins as chromophores. These are involved in a wide variety of essential photobiological reactions: (i) Photolyases, discovered more than 50 years ago, contain flavin adenine dinucleotide (FAD) as redox-active cofactor that initiates the reductive cleavage of thymine dimers in UV-light damaged DNA. (ii) The more recently discovered cryptochromes are related to the DNA-repair enzyme photolyase but operate either as UV-A/blue-light receptors or as central components of the circadian clock. They are important and ubiquitous sensor molecules that have been discovered in plants, animals, and more recently in cyanobacteria. Furthermore, they are currently the only candidate molecule for the magnetic compass of migratory birds and other animals based on radical-pair chemistry. (iii) The phototropin class of flavoproteins is another example in which

flavin mononucleotide (FMN) is involved as chromophore in the primary events of plant-stem bending towards a light source (phototropism), chloroplast relocation to places of appropriate light intensity, and the opening of stomatal guard cells to facilitate gas exchange. (iv) Finally, BLUF domains ("blue-light using FAD") mediate a light response in different molecular and cellular environments. In all these proteins, electron paramagnetic resonance (EPR) in all its flavors can be applied to study the fundamentals of their reaction mechanisms.
In this contribution an overview is given on our recent studies of various transient or stationary paramagnetic states (radicals, radical pairs, and triplet states) of photolyases, cryptochromes, phototropins, and BLUF domains, together with an outlook on how EPR can be exploited to gain a more general understanding of the fine-tuning of organic cofactors by their protein environment for their specific function.

IL122

Ultrafast spectroscopy on bimolecular photoinduced electron transfer reactions: new insight into old questions

Arnulf Rosspeintner[1], Marius Koch[1], François-Alexandre Miannay[1], Gonzalo Angulo[2], Bernhard Lang[1], Eric Vauthey[1]
[1]University of Geneva, Geneva, Switzerland; [2]Polish Academy of Sciences, Warsaw, Poland

Photoinduced electron transfer (ET) reactions are among the simplest photochemical reactions and, as such, have been intensively studied both theoretically and experimentally. Despite this, several important questions still remain unanswered.
One of them concerns the driving force dependence of bimolecular photoinduced ET that, contrary to most other types of ET processes, strongly departs from the predictions of Marcus theory. We have investigated the dynamics of these reactions as well as the nature of the primary product using a combination of ultrafast spectroscopic techniques. By looking at the early stages of the reaction, we could access the intrinsic ET rate constants and found that they are larger than the diffusion limit by several orders of magnitude. Moreover, a very weak, but distinct, inverted region is observed at high driving force. Using ultrafast transient IR spectroscopy, we could show that, at these driving forces, a new ET pathway leading to the ionic product in an electronic excited state opens up. Therefore, the weakness of the observed inverted region is not due to a failure of theory, but to the difficulty to identify the primary reaction product.
Another debated question concerns the occurrence of chiral selectivity in bimolecular ET, as the studies performed so far, using stationary or nanosecond-resolved techniques, gave very contrasted results and point to rather weak effects. We found that the situation is different when the intrinsic ET rate constant is considered. This was done by monitoring the early stages of the reactions, where the ET quenching occurs in the static regime and is not controlled by diffusion. This finding will be illustrated by recent results obtained with chiral helical fluorophores and chiral quenchers.

PL123

Danger signals and immunogenic cell death in PDT-induced anti-cancer immune responses

Abhishek D. Garg[1], Aleksandra Dudek[1], Gabriela B. Ferreira[2], Tom Verfaillie[1], Dmitri V. Krysko[3], Peter Vandenabeele[3], Chantal Mathieu[2], Patrizia Agostinis[1]
[1]Cell Death Research and Therapy (CDRT) Lab, Dept. Molecular Cell Biology, K. U. Leuven, Leuven, Belgium; [2]Laboratory for Experimental Medicine and Endocrinology (LEGENDO), K.U. Leuven, Leuven, Belgium; [3]Molecular Signalling and Cell Death Unit, Dept. Molecular Biomedical Research, VIB and Dept. Molecular Biology, Ghent University, Gent, Belgium

The main goal of anti-cancer therapies like photodynamic therapy (PDT) is to induce cancer cell death. Physiological cell death (i.e. tolerogenic apoptosis), induced by most anti-cancer therapies, is non-immunogenic or even actively immunosuppressive thereby compromising anti-cancer immune responses. However, recently it was found that cancer cell death elicited by certain modalities can be immunogenic. In fact, we recently characterized that hypericin-based PDT (Hyp-PDT) induces bona fide immunogenic cell death (ICD). ICD is characterized by spatiotemporally defined emission of immunogenic or danger signals like surface exposed (ecto-) calreticulin (CRT), ecto-HSP70, secreted ATP and passively released "chaperokines" (e.g. HSP90/70) and/or "endokines" (e.g. HMGB1). Together with tumour antigens these signals help in establishing a productive interface with the immune cells paving way for potent anti-cancer immune responses. Hyp-PDT induced ICD consists of a pre-apoptotic stage "rich" in both the numbers and amounts of simultaneously emitted danger signals (ecto-CRT, ecto-HSP70 and secreted ATP) – a unique feat. This, along with post-apoptotic release of chaperokines, elicits the uptake of dying cells, dendritic cell (DC) maturation, DC-based expansion of IFN-G producing CD4+/CD8+ T cells and tumour rejection responses, *in vivo*. Subsequently, following Hyp-PDT, ROS-based ER stress acts as an 'enabler' of cancer cell death (via PERK-based pro-death signalling) and ICD, such that abrogation of ER stress response (by depleting PERK) and ROS levels (by accentuating cellular anti-oxidant environment) compromises apoptotic cell death and emission of danger signals; to an extent that depletion of PERK suppresses the ability of Hyp-PDT to elicit tumour rejection, *in vivo*. In previous work, we showed that induction of autophagy along with ER stress acted as 'dampener' of Hyp-PDT induced apoptosis. To this end, unprecedented observations revealed that, Hyp-PDT-induced autophagy, but not blockage of caspase signalling, also acts as a 'dampener' of immunogenicity by assisting in suppression of crucial ICD determinants like ecto-CRT, DC maturation (and IL-6 production) and DC-based expansion of IFN-G producing CD4+/CD8+ T cells.
Thus, we have unravelled that Hyp-PDT is a potent inducer of ICD and anti-cancer immune responses (on certain levels, better than other ICD inducers). In this paradigm, ER stress acts as an 'enabler' while autophagy acts as a 'dampener' of immunogenicity and cell death. In future it would be crucial to translate these results (pre-)clinically.

PL124

Genetically-encodable fluorescent singlet oxygen photosensitisers

Santi Nonell
Institut Químic de Sarria - Universitat Ramon Llull, Barcelona, Spain

Fluorescent proteins are invaluable tools for fluorescence microscopy to monitor cellular processes in living cells, owing to their ability to be genetically-fused to virtually any protein in a cell. They are mostly used as tags, but their capability to act as singlet oxygen ($^1O_2$) photosensitisers is generating increasing interest in the context of electron microscopy, photodynamic therapy (PDT) and optogenetics.
Previous work from our laboratory has shown the ability of some variants from the green fluorescent protein (GFP) family to photosensitise $^1O_2$, although with very low efficiency owing to both the poor intrinsic efficiency of the chromophore but also to the limited access of oxygen to it. For example, TagRFP photosensitises $^1O_2$ with a quantum yield (Φ_Δ) of 0.004, similar to that of the free GFP chromophore. This notwithstanding, TagRFP is able to kill *E. coli* bacteria from the inside.
Efforts to produce genetically-encodable tags that generate $^1O_2$ have turned to the engineering of flavin mononucleotide (FMN)-binding fluorescent proteins, capitalising on the fact that the ubiquitous FMN is itself an efficient $^1O_2$ photosensitiser (Φ_Δ= 0.51). The first such photosensitising flavoproteins, termed miniSOG (for "mini Singlet Oxygen Generator") is a 15 kDa

flavoprotein with Φ_Δ = 0.03, one order of magnitude higher than that of TagRFP. Despite the relatively low yield, it dramatically outperforms TagRFP in the killing of bacteria. Moreover, cumulative irradiation of miniSOG increases its photosensitization ability by 10-fold due to a photoinduced transformation of the protein.

PL125
UV-B photoreceptor signalling

Roman Ulm
University of Geneva, Geneva, Switzerland

Plants are able to perceive ultraviolet-B radiation (UV-B) using the UV-B photoreceptor UV RESISTANCE LOCUS 8 (UVR8) which activates a specific molecular signaling pathway leading to UV-B acclimation. The UVR8 UV-B photoreceptor exists as a homodimer that instantly monomerises upon UV-B absorption via specific intrinsic tryptophans which act as UV-B chromophores. The UVR8 monomer interacts with CONSTITUTIVELY PHOTO-MORPHOGENIC 1 (COP1), an E3 ubiquitin ligase, initiating a molecular signaling pathway that leads to gene expression changes. This signaling output leads to UVR8-dependent responses including UV-B-induced photomorphogenesis and the accumulation of UV-B-absorbing metabolites that function as "sunscreens". Negative feedback regulation of the pathway is provided by the WD40-repeat proteins REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2, which facilitate UVR8 redimerization, disrupting the UVR8-COP1 interaction. I will present our latest understanding of the biological process in plants from initial UV-B perception and signal transduction through to the known UV-B responses that promote survival in sunlight.
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PL126
Subcellular control of signaling pathways with nanoparticles and light

Mathieu Coppey
CNRS UMR168 Unite physico-chimie Curie (P.C.C), Paris, France

The cell architecture and dynamics are controlled by a complex molecular circuitry able to process information from environmental cues in order to drive the cell into functional states accordingly. For instance, cells get polarized as they migrate or divide. To do so, they have to amplify local and transient signals into stable and system-level asymmetries. To understand how molecular events within the protein interaction network can be coordinated up to the emergence of cell functions there is a clear need for experimental approaches which allows perturbations of the signaling network at a spatial and temporal resolution sufficient to match the timing and extent of subcellular protein dynamics.
I will present two emerging classes of genetically encoded experimental tools which allow the activation of intracellular signaling pathways with an unprecedented spatial and temporal resolution. First I will discuss the "magnetogenetic" approach based on the use of functionalized magnetic nanoparticles. Then I will discuss the "optogenetic" approach which relies on a light-switchable dimerization to gate the activity of a protein. I will use the canonical RhoGTPase pathway which control cell polarity as an example to illustrate practically these perturbative approaches.

IL127
Biomarkers of photo-induced damage to DNA: applications to repair studies

Jean Cadet, Thierry Douki
INAC CEA/Grenoble, Grenoble, France

Exposure of cellular DNA to solar radiation gives rise to mostly bipyrimidine lesions including predominant cyclobutane pyrimidine dimers (CPDs), pyrimidine (6-4) pyrimidine photoproducts (6-4PPs) and their valence Dewar isomers (DewPPs) with a distribution for each class of photodamage that is strongly primary sequence dependent. In addition UVA radiation that is able to induce CPDs through direct interaction with DNA bases has been found to trigger photodynamic effects through the photosensitized guanine (8-oxoGua) is generated as the main oxidation product together with lower amounts of oxidized pyrimidine bases and single strand breaks (SSBs) at the exclusion however of double strand breaks. Due to the high mutagenicity of several DNA photoproducts, mostly those generated at CC and TC sites and their likely involvement in skin cancer development, efforts have made to monitor the formation of the main classes of photo-induced DNA damage in isolated cells and skin. Among the most successful approaches already initially designed more than 30 years ago and still widely used one may mention the preparation of polyclonal and monoclonal antibodies against CPDs, 6-4PPs and DewPPs. These exhibit enough specificity to detect each class of bipyrimidine photoproducts as radioimmunoassays, ELISA and immuno-dot-blot assays. Other relevant applications deal with immunostaining that allows the localization by fluorescence detection of bipyrimidine photoproducts in the skin. The global measurement of CPDs has been also achieved with a high sensitivity in UV-irradiated cells upon DNA incubation with bacterial T4 endonuclease V and subsequent measurement of induced SSBs by either alkaline elution technique or the alkaline comet assay. However the latter biochemical assays are not able to distinguishing between the different positions isomers of given dimeric lesions and also provide only semi-quantitative information due to the lack of internal calibration. The availability of accurate and quantitative HPLC coupled to tandem mass spectrometry method operating in the electrospray ionization mode (HPLC-ESI-MS/MS) allows overcoming these limitations. Thus the twelve possible dimeric pyrimidine photoproducts have been measured in the DNA of isolated cells and biopsies from human skin explants after exposure to relatively low doses of UVB, UVA or solar radiations. The HPLC-MS/MS measurements allow the determination of the rate of removal of each of the photoproducts through the global nucleotide excision repair pathway. Thus differences were observed between the different classes of bipyrimidine photoproducts and also among the four possible types of CPDs. One may also mention the detection of CPDs and 6-4PPs at the nucleotide level by ligation-mediated PCR, a method that however suffers from a lack of sensitivity. HPLC analysis with electrochemical or MS/MS detection has been used for monitoring the formation of 8-oxoGua in UVA-irradiated cells and skin. Enzymatic assays based of the use of formamido-pyrimidine DNA glycosylase and endonuclease III for revealing 8-oxodGuo and oxidized pyrimidine bases constitute suitable alternatives.

IL128

Reaction mechanisms of DNA photolyases with special consideration of new results on repair of the (6-4) photoproduct

Klaus Brettel

Laboratoire Mécanismes Fondamentaux de la Bioénergétique, UMR 8221, CEA-iBiTec-S, CNRS, Université Paris Sud, Gif-sur-Yvette, France

UV irradiation induces two major types of harmful crosslinks between adjacent pyrimidines in DNA: cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone photoproducts ((6-4) photoproducts). In many organisms, these lesions are repaired by photolyases [1,2], flavoproteins that require light for their catalytic action. The repair mechanism involves electron transfer to the lesion from the photoexcited state of the fully reduced flavin cofactor $FADH^-$, splitting of the intra-dimer bonds and return of the excess electron to the flavin. An overview will be given of advances on the kinetics and mechanism of CPD repair [3] and on intra-protein electron transfer for formation of the catalytically active fully reduced $FADH^-$ state ("photoactivation") [4,5], to which the author's lab contributed also by developments in transient absorption spectroscopy [6-8].

Repair of the (6-4) photoproduct is chemically more challenging and less well understood than repair of the CPD. New experimental data [9] will be presented that demonstrate that nature resorts to a two photon mechanism for repair of the (6-4) photoproduct.

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IL129

Unexpected roles of mutagenic translesion synthesis and mismatch repair in responses to ultraviolet light

Anastasia Tsaalbi-Shtylik[1], Jacob G. Jansen[1], Frank R. de Gruijl[2], Niels de Wind[1]

[1]Department of Toxicogenetics, Leiden University Medical Center, Leiden, Netherlands; [2]Department of Dermatology, Leiden University Medical Center, Leiden, Netherlands

The maintenance of genomic integrity upon exposure to UV light depends on nucleotide excision repair (NER), that repairs DNA photolesions, and translesion synthesis (TLS) that performs replicative bypass of photolesions that have escaped repair. Although TLS enables the completion of replication at damaged DNA templates, it is an error-prone process that is responsible for most DNA damage-induced mutations. To study the role of mutagenic TLS in the development of skin cancer we have used mice deficient for the NER gene *Xpc* that, in addition, carry a hypomorphic allele of the TLS gene *Rev1*, involved in TLS of (6 4)pyrimidine-pyrimidone photolesions [(6 4)PP]. Indeed, UV-induced mutagenesis was reduced in *XpcRev1* cells and mice, compared with the *Xpc* controls. Conversely, DNA damage signaling was increased in the *XpcRev1* mutant, presumably as a consequence of the transient replicative stress at (6 4)PP. Despite the reduction in mutagenesis, carcinogenesis was accelerated in the *XpcRev1* mutant, compared with the *Xpc* controls. Analysis of UV-exposed *Rev1Xpc* skins revealed a severe, NF-κB and Interleukin-6-mediated hyperplasia that could be inhibited with a variety of anti-inflammatory compounds. Thus, mutagenic TLS unexpectedly suppresses skin carcinogenesis by modulating the balance between cancer initiation (mutagenesis) and replicative stress-dependent tumor promotion (i.e. proliferation).

Canonical DNA mismatch repair (MMR) repairs misincorporations during replication of undamaged DNA templates. However, MMR proteins also play, poorly-defined, roles in cellular responses to UV light. This involvement was studied using isogenic cells with individual and combined targeted defects in NER, TLS and MMR proteins. We found that the MMR protein Msh2/Msh6 reduces the mutagenicity of low-dose UV light while inducing DNA damage responses, including cell cycle checkpoints and apoptosis. Using a variety of genetic and biochemical approaches we have unveiled a new MMR-related excision repair pathway, dubbed damage-response MMR (drMMR), that excises misincorporations introduced by mutagenic TLS opposite unrepaired DNA photolesions. While drMMR mitigates the mutagenicity of TLS, persistent drMMR-induced excision tracks induce DNA damage signaling and concomitant cell cycle arrests. In conclusion, our data demonstrate that NER, TLS and MMR play unanticipated roles in defining mutagenic, cell cycle, inflammatory and carcinogenic responses to DNA photolesions.

IL130

PARylation: A novel player in the repair of UV-damaged DNA

Girish M. Shah, Rashmi G. Shah, Nupur K. Purohit, Mihaela Robu

Laval University and CHU-Q (CHUL) Research Centre, Lab Skin Cancer Research, Québec City, Québec, Canada

PARylation is a post-translational modification of target proteins by polymers of ADP-ribose (PAR) that are formed by catalytic activity of the members of poly(APDP-ribose) polymerases (PARP) family of proteins. The principal member of this family PARP-1 is very rapidly activated in response to different types of DNA damages to PARylate itself and other target proteins in the vicinity of DNA damage. The PARylation transiently alters functions of these proteins until PAR is degraded and proteins are restored to their native state. This rapid reaction to DNA damage has been implicated in various responses of mammalian cells ranging from DNA repair to death. Although PARP-1 is activated in UVB or UVC-irradiated cells and PARP inhibitors potentiate UVB-induced skin carcinogenesis, the significance of PARP-1 and PARylation specifically in repair of UV-induced DNA damage did not receive much attention until recently. UV irradiation causes two types of DNA damages which are repaired by two distinct DNA repair pathways: (i) the direct DNA photolesions, such as cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts are removed by the nucleotide excision repair (NER) pathway; and (ii) the indirect or oxidative DNA damages are eliminated by base excision repair (BER) pathway. While the role of PARP-1 in BER is well known, most recent studies from our group and other teams have identified a novel role of PARP-1 in mammalian NER. We will present published and novel evidence demonstrating the role of PARP-1 and PARylation in collaboration with some of the key NER proteins in the lesion recognition step of the global genomic sub-pathway of NER that repairs the lesions from the entire genome. Finally, we will provide a perspective for a much larger role for PARP-1 and PARylation in other steps of NER. Our studies could pave the

way for identification of other factors implicated in the repair of UV-induced DNA lesions uniquely in higher organisms.

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IL131

Genes that may be important in skin cancer: comparison of UVB and UVA1 responses in human skin in vivo

Antony Richard Young, Robert Sarkany, Angela Tewari

King's College London, London, United Kingdom

We lack data on the spectral dependence of skin cancer in human skin. We have compared erythemally equivalent doses of UVB (300 nm) and UVA1 (340-400 nm) on gene expression by RTPCR, after initial screening by whole genome microarray. At such doses, there are about 3-4 times more UVB-induced epidermal cyclobutane pyrimidine dimers (CPD) when assessed immediately post-UVR. We report here on some genes that may have relevance for different stages of skin cancer.

UVA1 and UVB up-regulate NER and BER genes predominantly at 24h *vs* 6h. Induction of BER appears to be 13% greater than NER (24h) by both UVA1 and UVB, which suggests that oxidative damage to DNA is better repaired *in vivo*. The degree of up-regulation of NER and BER is approximately the same for both wavebands, but there may be important differences within the epidermis. Overall, the repair kinetics of epidermal CPD after 3MED UVB (90mJ/cm^2) and UVA1 (148 J/cm^2) is the same. However, there are important differences in the basal layer in which UVA1-induced CPD in keratinocytes and melanocytes are poorly repaired compared with those induced by UVB.

Disruption of the extra-cellular matrix is likely to be important in skin cancer. UVA1 induces similar levels of epidermal matrix metalloproteinase-1 (MMP1) as UVB but about 3 fold more in the dermis at 24h. We have observed that MMP12 (macrophage elastase) (RNA, protein and enzyme activity) expression is more sensitive to UVA1 than UVB. Mice lacking elastases such as neutrophil elastase are less prone to UVR-induced squamous cell carcinoma.

UVR-induced alteration of skin immunity is also thought to be important in skin cancer. IL10 (and other immunoregulatory factors) are similar with UVA1 and UVB, despite the differences in levels of CPD. This suggests that UVA1 induced CPD may be eliciting more of an immunoregulatory response for a given number of CPD, but chromophores other than DNA may be important.

Overall, our data raise concern about the carcinogenic potential of UVA1 (>70% of solar UVR) which may be greater than that suggested by animal studies.

OC132

The Role of Brm in Non-Melanoma Skin Cancer

Andrew William Farrell

The University of Sydney, Sydney, NSW, Australia

Australia has the highest incidence of non-melanoma skin cancer (NMSC) in the world. These include malignant squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) as well as actinic keratosis (AK). These cancers are predominantly caused by exposure to ultraviolet (UV) radiation in sunlight. The mammalian chromatin remodelling complex SWI/SNF is known to modulate transcription and DNA repair in response to UV damage. The Brm subunit of the SWI/SNF complex is one of two mutually exclusive subunits that provides energy for remodelling and negatively regulates cellular proliferation. Our studies have shown that Brm knockout mice have a greater incidence of UV-induced skin cancer as compared to wildtype controls. It has also been shown that Brm is lost in the progression of benign AK into malignant SCC, suggesting loss of Brm correlates with invasion or progression. Considering this loss of Brm, NMSC samples were screened for sequence variants and it was found that the same single base substitution occurred in approximately 35% of BCCs and 10% of SCCs. This G:C to T:A transversion resulted in substitution of glutamine by lysine at codon 203 (Q203K). This demonstrates a previously unidentified Brm mutation in 17% of studied NMSC and is the first study to detect mutations in this gene for any type of cancer. Upon UV irradiation, Brm knockout keratinocytes recover prematurely from cell cycle arrest, likely allowing the proliferation of cells that have not had sufficient time to repair their damaged DNA. This project further aims to study the function of the Q203K mutation as compared to the loss of Brm in key aspects of keratinocyte responses to UV-induced DNA damage such as proliferation, UV-induced apoptosis, DNA repair, photolesion formation and invasion. However the data indicates that Brm appears to be a tumour suppressor gene that protects skin keratinocytes from the damaging effects of UV radiation. In the absence of Brm, UV irradiated cells have inhibited UV-induced cell cycle arrest, leading to increased proliferation of keratinocytes after UV radiation. This may enhance mutation fixation from UV damaged DNA.

OC133

Preclinical Corrective Gene Transfer in Xeroderma Pigmentosum Human Skin Stem Cells

Thierry Magnaldo[1], Emilie Warrick[1], Francoise Bernerd[2]

[1]CNRS, Nice, France; [2]L'Oreal, Clichy, France

Xeroderma pigmentosum (XP) is a devastating disease associated with dramatic skin cancer proneness. XP cells are deficient in nucleotide excision repair (NER) of bulky DNA adducts including ultraviolet (UV)-induced mutagenic lesions. Approaches of corrective gene transfer in NER-deficient keratinocyte stem cells hold great hope for the long-term treatment of XP patients. To face this challenge, we developed a retrovirus-based strategy to safely transduce the wild-type XPC gene into clonogenic human primary XP-C keratinocytes. *De novo* expression of XPC was maintained in both mass population and derived independent candidate stem cells (holoclones) after more than 130 population doublings (PD) in culture upon serial propagation. Analyses of retrovirus integration sequences in isolated keratinocyte stem cells suggested the absence of adverse effects such as oncogenic activation or clonal expansion. Furthermore, corrected XP-C keratinocytes exhibited full NER capacity as well as normal features of epidermal differentiation in both organotypic skin cultures and in a preclinical murine model of human skin regeneration in vivo. The achievement of a long-term genetic correction of XP-C epidermal stem cells constitutes the first preclinical model of ex vivo gene therapy for XP-C patients.

OC134

Quiescent stem cells targeted in skin carcinogenesis by low level UV exposure

Gerline C van de Glind, Marika J van Kempen, Heggert G Rebel, Cornelis P Tensen, Frank R. de Gruijl

LUMC Department of Dermatology, Leiden, Netherlands

UV radiation is a major risk factor in the development of skin cancer. It induces cyclobutane pyrimidine dimers (CPDs) in the DNA, which at low level UV exposures – in absence of hyperplasia - can accumulate in quiescent stem cells. When these cells are forced to proliferate mutations may occur. The aim of this study was to investigate if such a forced proliferation gives rise to p53-mutant overexpressing clones and ultimately skin carcinomas.

Materials and methods: CPD-retaining epidermal cells were induced in SKH mice by 40 days of 0.14 MED/day. A control group of mice received the same total UV dose (5.6 MED) in a single exposure on day 1; this regimen will not result in an accumulation of CPDs. 47 days after the start of the experiment both groups received TPA treatment to induce cell proliferation

for up to 20 weeks. IHC stainings were performed for CPDs and P53 patches. Tumours were registered and mapped for each mouse .

Results: After 40 days sporadic CPD-retaining cells (1.3% of basal cells) were only detected in the mice that received 0.14 MED/day. Before TPA treatment these cells were mostly observed in the basal layer. After TPA treatment CPDs were observed in the suprabasal layers. After 8 weeks of TPA treatment p53-overexpressing foci were only observed in the low-level exposure group (1 focus per 5000 CPD-retaining basal cells). Tumour free survival for 2 and 4 mm "non-papilloma" tumours (endophytically growing suspected carcinomas and precursing actinic keratoses) was significantly longer in the high-level exposure group. For frank papillomas (exophytically growing) there was no significant difference. Non-papilloma tumours went into regression after discontinuation of TPA in the group that received a single high UV dose, but persisted and increased in the group that received the low-level exposure over 40 days (0.06 vs 2.25 tumours >4mm/mouse after 280 days).

Discussion: Our data indicate that quiescent stem cells are targeted in skin carcinogenesis by low level UV exposure.

IL135

Nano-photosensitizers with enhanced efficiency for two-photon photodynamic therapy

Qing-Hua Xu

National University of Singapore, Singapore, Singapore

Photodynamic therapy (PDT) is a promising non-invasive treatment of cancers or other diseases. Two-photon PDT is advantageous over the traditional one-photon PDT in a few aspects such as 3-D selectivity and deep penetration into diseased tissues. Photosensitizers are essential materials in PDT. However, most photosensitizers in clinical use have been optimized for the conventional one-photon PDT and have very small two-photon absorption cross sections. Their efficiencies for two-photon PDT are generally low. To make two-photon PDT more widely applicable, it is essential to develop photosensitizers with large two-photon absorption cross sections. In this talk, I will present our recent work on development of composite nano-photosensitizers with enhanced two-photon excitation properties. We used two different strategies to improve two-photon excitation efficiency of photosensitizers. One method is based on energy transfer from conjugated polymers that have large two-photon absorption cross sections to photosensitizers. We have used conjugated polymers as two-photon light harvesting materials to improve the singlet oxygen generation efficiency of photosensitizers and cancer cell killing efficiency by up to hundreds of times, and eventually developed composite photosensitizers with dual capability of two-photon imaging and PDT. The second approach is based on plasmon resonance enhancement. Noble metal nanoparticles are known to display interesting phenomenon called Plasmon resonance, which could be utilized to enhance the optical properties of nearby chromophores. We have developed nanocomposites containing gold nanorods and photosensitizers, which displayed significantly enhanced two-photon excitation efficiency and two-photon PDT activity over cancer cells.

IL136

Nanoparticle-photosensitizer dyads for biological photosensitization

Benjamin Ehrenberg, Elina Haimov, Sara Houpt

Bar Ilan University, Dept. of Physics, Ramat Gan, Israel

We introduce the use of existing, as well as novel, nanoparticles as carriers of photosensitizers and as enhancers of their photophysical activity. Pdots, a new type of nanoparticles, have the promise of serving many biophysical applications. They are nanometer-sized particles, composed of semiconducting organic dye polymers. They are processed by mixing them with various block copolymers, forming by sonication colloidally very stable particles, whose water solubility can be tuned by pegylation. We employ Pdots in biological photosensitization to load and carry active photosensitizers for treatment and diagnostics in PDT, and to enhance their activity by serving as light-collecting "antennas". Pdots can adsorb photosensitizer molecules by non-covalent mechanism. They possess broad absorption bands in the visible range and have very bright fluorescence spectra. At close proximity between the particle and the sensitizer, FRET occurs between the Pdot as the donor, and the sensitizer as the acceptor. Several types of organic polymers can be chosen, so that their narrow emission band will overlap the longest wavelength absorption band of the photosensitizer. By employing this methodology, one overcomes the usually weak light absorption of the sensitizers and the efficiency of photosensitization can be enhanced. In a similar way, quantum dots can be processed to make them water-soluble and good non-covalent binders of amphiphilic photosensitizers. The presentation will show preparation procedures, spectroscopic properties, FRET efficiencies to photosensitizers, enhanced photosensitization in solution, uptake of the dyads by cells and their biological photosensitization efficiency.

IL137

Silencing photosensitizers through nanoencapsulation

Norbert Lange

University of Geneva, School of Pharmacy, Laboratory of Pharmaceutical Technology, Geneva, Switzerland

Since recently, targeted and non-targeted nanoparticular delivery systems as vehicle for photoactive compounds have attracted particular attention in photodynamic therapy and fluorescence diagnosis. Biodegradable systems combine several advantages including the possibility to formulate lipophilic compounds, increase the plasma half life, reduce the binding to plasma proteins, hide potential intrinsic toxicity, and graft additional targeting moieties to further enhance the selectivity achieved through the enhanced penetration and retention effect.

However, care must be taken with respect to size selection, polydispersity and, and drug loading of these nanoparticles for efficient delivery of photoactive compounds. In this presentation parameters influencing the photophysical properties of photosensitizer encapsulated into nanoparticles will be discussed.

IL138

Interactions between photosensitizers and biomembranes

Mariusz Kępczyński

Jagiellonian University, Faculty of Chemistry, Kraków, Poland

Natural or synthetic porphyrins are being used as photosensitizers in photodiagnosis (PD) and photodynamic therapy (PDT) of malignancies and some other diseases. Understanding the interactions between the porphyrins and cell membranes is therefore important to rationalize the uptake of photosensitizers and their passive transport through cell membranes. Liposomes (artificial membranes) are widely used in general investigations of passive drug uptake, especially in the study of photosensitizers for use in PDT.

Liposomes containing lipids with covalently attached poly(ethylene glycol) (PEG) are known as pegylated or sterically stabilized liposomes (SSL) and can be applied as effective delivery vesicles for photosensitizers. On the other hand, the pegylation of photosensitizers can significantly change the tumor-to-normal tissue ratio (TTR), which is a measure of the preferential accumulation of the photosensitizer in the malignant tissue.

In this presentation, I consider the properties of hematoporphyrin (Hp, a well-known photosensitizer for PD and PDT), 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (m-THPP) and its pegylated derivative in the presence of a lipid bilayer, used as a model system for protein-free cell membranes, and a pegylated

membrane. The interactions between the porphyrins and membranes were studied using fluorescence methods and computer simulations. The fluorescence methods are useful to determine the affinity of a given photosensitizer to accumulate in the lipid bilayer and its depth localization inside the membrane. Atomic-scale molecular dynamics (MD) simulations allow one to estimate the position, orientation, and dynamics of the porphyrin molecules inside the membrane and its tendency to migrate from one leaflet to the other (flip-flop movements).

OC139

A rationale for the development of heterogeneous singlet oxygen photosensitizers

Yair Ezequiel Litman[1], Hernán Bernardo Rodríguez[2], Silvia Elsa Braslavsky[3], Enrique San Román[1]

[1]*INQUIMAE/DQIAyQF, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina;* [2]*INIFTA, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina;* [3]*Max-Planck-Institut für Chemische Energiekonversion, Mülheim an der Ruhr, Germany*

As a general rule, dyes with a high triplet quantum yield are required for the development of photosensitizers based on the production of reactive oxygen species suited for photodynamic therapy and related applications. Though molecular photosensitizers are currently used, some applications – e.g., bacterial inactivation – can be favored by the use of heterogeneous systems like nanoparticle suspensions, microparticle beads or immobilized films. For these systems to be effective, high light absorption rates, achievable at high dye concentrations, are mandatory. Unfortunately, at the required dye concentrations, molecular aggregation and formation of statistical traps usually lead to singlet state deactivation with the consequent loss of triplet yield.

During the last years we have developed various methods to account quantitatively for the photophysics of dyes embedded into light scattering solid materials. In particular, recently we determined triplet quantum yields of dyes in model systems as a function of concentration using Laser Induced Optoacoustic Spectroscopy (LIOAS). Whereas phenazinium dyes showed a rapid decrease of triplet formation with concentration, various xanthene dyes yielded practically constant triplet quantum yields up to equivalent bulk concentrations in the order of 10^{-3} M.

A radical pair recombination mechanism based on the charge transfer quenching of the singlet state by neighboring dye molecules may account for this behavior. The triplet state quantum yield decreases in parallel to the fluorescence quantum yield for dyes in which the energy of the charge transfer state lays below the energy of the dye pair triplet state, whereas fluorescence concentration quenching with triplet formation takes place in the opposite case. As excitonic interactions depend on the square of the transition moment, they are negligible for triplet dye pairs, which behave as isolated, monomeric triplets.

These results provide a strong rationale for the design of heterogeneous photosensitizers with high triplet quantum yields at large dye concentrations.

OC140

Resistance of the membranes to the photodynamic effect: stress-relaxation pathways governed by the physical and chemical parameters

Rachid Kerdous, Aurélien Bour, Stéphanie Bonneau

Université Pierre et Marie Curie, Paris, France

Membranes lipids are important targets of the photodynamic effect, as well as for photodynamic therapy than for photochemical internalization. Their chemical modifications under photo-oxidation have extensively been studied, involving major modifications within the membranes which can be highly destabilized. Here, our purpose is to demonstrate that the photo-induced permeabilization of the membranes is correlated with a deep physical stress, which can be relaxed by various pathways, depending on its lipidic composition.

Using Giant unilamellar vesicles, we asymmetrically or symmetrically oxidize the membranes. We observed different shape transitions such as oblate to prolate and budding, which are typical of membrane curvature modifications. The asymmetry of the shape transitions is in accordance with a lowered effective spontaneous curvature of the leaflet being targeted. We interpret this effect as a decrease in the preferred area of the targeted leaflet compared to the other, due to the secondary products of oxidation (cleaved-lipids). Permeabilization of giant vesicles by light-induced oxidation is observed after a lag and is characterized in relation with the photosensitizer concentration. We interpret permeabilization as the opening of a pore above a critical membrane tension, resulting from the budding of vesicles. The evolution of photosensitized giant vesicle lysis tension was measured and yields an estimation of the effective spontaneous curvature at lysis. Additionally photo-oxidation was shown to be fusogenic.

In relation with this physico-chemical scenario, several types of membranes were studied, and the effects of their composition and their subsequent properties were determined. In particular, the cholesterol, able to flip quickly, offers to the membrane a cholesterol-mediated stress relaxation pathway. The cardiolipine, given to the membrane 2D-nematic properties, contribute to its higher resistance to oxidation.

OC141

Porphyrin-triphenylamine hybrids for 2PA-PDT: Synthesis, photophysical properties and biological applications

Guillaume Garcia[1], Florent Poyer[2], Fabien Hammerer[1], Philippe Maillard[1]

[1]*Institut Curie / CNRS UMR176, Orsay, France;* [2]*Institut Curie / INSERM U759, Orsay, France*

Introduction: The most common photosensitizers (PS) currently used in PDT have one-photon-absorption (1PA) peak in the visible wavelength range (400-700 nm) but an important limitation arise from the fact that the penetration depth at these wavelengths is confined near the surface of the tissues. One way to overcome this problem consists in two-photon absorption (2PA). 2PA-PDT should allow also greater precision than is achievable by conventional 1PA. Another limitation of PDT is the low selectivity and specificity of the PS for tumor cell: high drug and light doses are required to compensate this, leading to damage of healthy tissues. Active targeting of membrane receptors represents an obvious improvement.

Method and Results: The strategy used in this work consists in conjugated porphyrins triphenylamine hybrids synthesis. In these structures, a cooperative effect has been demonstrated: these compounds exhibit interesting 2PA cross section combined with high singlet oxygen quantum yield. Water soluble methylated hybrids were also synthesized and studied. Moreover, we introduce sugar moieties to interact with specific receptors at the surface of some cancer cells. Internalization, localization and phototoxicity of these porphyrin derivatives were studied.

Conclusion: We describe the synthesis of porphyrin triphenylamine hybrids designed as 2PA-PDT agents. The targeted analogous, substituted by three sugars on each chromophore, exhibit high two photon absorption cross sections and high singlet oxygen quantum yields making of this ones potential two-photon excited photosensitizers.

OC142

Conjugated porphyrin dimers as concurrent photosensitizers and viscosity probes

Maryam Qurashi

Imperial College London, London, United Kingdom

Photodynamic therapy (PDT) is the use of light-absorbing molecules to produce singlet oxygen, involved in the treatment of malignant tissues. This is achieved when singlet oxygen initiates damaging side reactions which may oxidise lipids and proteins and produce aberrations in their natural structures. It has been hypothesized that oxidative damage in the cell membrane can cause crosslinking of unsaturated lipid chains; increasing the rigidity of the membrane and interrupting the biochemical processes[1–3]. Similarly, singlet oxygen damage in the cytoplasm oxidises the hydrophilic surfaces of proteins and causes their aggregation, increasing their viscous drag on other diffusing molecules. This increase in viscosity, in turn reduces the rate of macromolecular diffusion, and so restricts the interactions of the cytotoxic singlet oxygen[4]. As such, measurement of cellular viscosity before and during PDT will allow us to evaluate the dynamic relationship between viscosity and efficient PDT. We have already shown that the apparent viscosity of SKOV-3 cells increases as they are irradiated, as a result of increased singlet oxygen production[4]. Here, we investigate further the mechanism of viscosity sensing and the possibility of viscosity increase as a precursor to cell death during PDT. The dual functionality of the probe allows us to study viscosity and initiate PDT with one molecule.

For this purpose, we have developed a porphyrin-based photosensitizer that belongs to a group of viscosity-sensitive fluorescent molecules, termed 'molecular rotors'[4–6]. Emissive properties of molecular rotors are sensitive to the viscosity of their surrounding medium. The diversity of cellular domains and organelles implies spatially and temporally dynamic viscosity which is best described by imaging it in vivo. Such imaging is made possible by the use of biocompatible, fluorescent molecular rotors, which, through a mechanism predicted by the Förster-Hoffman equation, are capable of reporting a large range of solution viscosities[4–6].

This presentation discusses a fluorescent molecular rotor, constructed as a conjugated porphyrin dimer. Two zinc-porphyrin macrocycles rotate around the axis of a butadiene link, adopting one of two spectrally distinct emissive states. Rotation is viscosity dependent, and thus, the ratio of these two states can be correlated with viscosity, this is termed ratiometric imaging. Ratios of emission intensity are concentration independent, allowing us to take viscosity measurements from anywhere in the cell that the dye can be localised[4,7]. Additionally, the two confomers have vastly different singlet oxygen quantum yields, in this way we can isolate the production of singlet oxygen and the sensing of viscosity in time[8].

Additionally, due to its high degree of conjugation, the dimer has an extremely high two-photon absorption cross section (14000-17000 GM for some charged variants of the dimer)[9]. Two-photon-absorption is a non-linear process where two photons are simultaneously absorbed by a molecule, resulting in a small focal volume compared with single photon excitation. A small focal volume is a vastly advantageous tool during imaging as it confers high spatial resolution, and in our case a simultaneously high area of specificity for PDT irradiation. Additionally, two-photon-absorption occurs at deeper penetrative distance into tissues and is ideal for the application of photodynamic therapy where irradiation of healthy tissue is to be avoided.

Finally, we present here a novel photosensitizer which has the capability to report the effects of PDT upon intracellular viscosity and, in turn, the viscous effect on singlet oxygen diffusion. We have already collected promising data in the area of viscosity imaging during PDT and are further investigating the nature of viscosity sensing in different locales of the cell such as the membrane. We aim to investigate the causes of viscosity increases during PDT by imaging the process with by using a single non-endogenous dye.

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OC143

Influence of external bacterial structures in the efficiency of photodynamic inactivation

Marlene Pereira, Maria Amparo Faustino, João Tomé, Graça Neves, Augusto Tomé, José A. S. Cavaleiro, Ângela Cunha, Newton C. M. Gomes, Adelaide Almeida

University of Aveiro, Aveiro, Portugal

Photodynamic inactivation (PDI) is receiving considerable attention for its potential as a new form of bacterial inactivation. The main targets of PDI are the external structures, cytoplasmic membrane and cell wall. The aim of this work was to evaluate the influence of the external bacterial structures in the efficiency of PDI. To reach this objective a tetra-cationic porphyrin (Tetra-Py^{+}-Me) at 5.0 μM, was tested against 8 bacteria with distinct external structures, 4 Gram-negative (G-) bacteria (*Escherichia coli*, with typical Gram-negative external structures; *Aeromonas salmonocida*, *Aeromonas hydrophila* both with a S-layer instead of a peptidoglycan layer and *Rhodopirellula* sp., with a S-layer and with compartmentalization of the cytoplasm) and 4 Gram-positive (G+) bacteria (*Staphylococcus aureus*, with typical G+ external structures; *Truepera radiovictrix*, *Deinococcus geothermalis* and *D. radiodurans*), all with a thick cell walls that give them G+ stains, but including a second complex multi-layered membrane and so are closer in structure to those of G- bacteria, upon white light at 4.0 mW cm^{-2} for 270 min. Cellular uptake of PS by each bacteria was also determined using the same PS concentration. In the presence of PS, the viability of bacteria was affected according to the bacterial characteristics. G+ bacteria were more efficiently inactivated than G- ones. Although all G+ bacteria were inactivated to the detection limit (8 log), the PDI followed distinct patterns (*e.g.* reductions after 30 min of ~ 4 log for *T. radiovictrix* and *D. geothermalis*, ~ 6 log for *S. aureus* and ~ 7 log for *D. radiodurans*). Among the G- bacteria, *E. coli* was the only species to be inactivated to the detection limit (~ 8 log after 180 min). The PDI efficiency of the

two *Aeromonas* strains was similar (reduction of ~ 5-6 log after 270 min). The *Rhodopirellula* was the least susceptible to PDI (reduction of ~ 4 log after 270 min). The cellular uptake of PS by the bacteria varied proportionally to the inactivation, being similar (1.2-1.8 x 10^6 molecules $cell^{-1}$) for all G+ strains and varying between 1.0 x 10^5 (*Rhodopirellula*) and 4.5 x 10^5 (*E. coli*) molecules $cell^{-1}$ for G- strains. The G+ bacteria were more easily inactivated than G- and this was even true for *T. radiovictrix*, *D. geothermalis* and *D. radiodurans*, which have a complex multi-layered cell wall with 5-6 layers. Although the results show that the composition of the cells coating is a major determinant of the susceptibility of bacterial cells to PDI, further studies are still necessary to clarify the underlying mechanisms of the PDI process.

OC144

Design of nanoparticles for amplifying singlet oxygen production

Sara Mooi, Belinda Heyne

University of Calgary, Calgary, Alberta, Canada

In the past decade the metal enhancement effect has been investigated for its unique influence on fluorescence emission, and increase in fluorescence intensity is readily observed for a fluorophore in close proximity to a metal nanoparticle. On the contrary, studies involving enhancement of singlet oxygen production by metal colloids are relatively scarce and so far only stationary silver island films have been proven to be adequate to do so. The application of metal enhancement effect in photodynamic therapy is therefore limited since the metal colloids are anchored onto glass or polymeric substrates in the case of silver island films. In the study presented herein, we have engineered novel nanoparticles based on a core-shell approach on which a photosensitizer has been covalently tethered to the nanoparticle shell. As a proof-of-concept, we developed a silver nanoparticle coated with a silica shell decorated with Rose Bengal. These nanoparticles were not only able to generate singlet oxygen, but singlet oxygen production was greatly amplified. Our results indicate these novel nanoparticles have a singlet oxygen quantum yield greater than one. This unusual quantum yield was explained in term of a synergistic effect in which excitation of RB occurs simultaneously through direct irradiation and indirect plasmonic effects. These nanoparticles open the doors to new nanotechnologies to be used in photodynamic therapy.

IL145

Extracorporeal Photopheresis State of the Art 2013

Robert Knobler

Department of Dermatology, Medical University of Vienna, Vienna, Austria

Extracorporeal photoimmunotherapy (ECP, photopheresis), was first introduced at the department of dermatology, Columbia Universtiy by R. Edelson and co-workers. in 1982, receiving shortly thereafter FDA approval for its palliative use in the treatment of CTCL Over one million treatments later, in 2013, numerous studies across the world have provided important leads for the better understanding of its key mechanisms of action (apoptosis, induction of regulatory T-cells, GvHD equivalent animal models, etc.) as well as increased documentation for potential major new indications: treatment and possible prevention of acute and chronic Graft versus Host disease after allogeneic bone marrow transplantation, control or possible prevention of rejection in the area of solid organ transplantation (Heart, Lung, Face, Liver, Kidney), systemic sclerosis, and other T-cell mediated diseases including Chron's disease, ulcerative colitis, nephrogenic fibrosing dermopathy (NFD), refractory atopic dermatitis and localized scleroderma among others. With the introduction of the FDA and EC approved use of the extracorporeally applied form of liquid psoralen (UVADEX) the technology has been able to deliver improved efficacy, reproducibility, and reliability associated with a very low side effect profile. New developments have also made this therapy more available to low body weight patients (e.g.: children). Photopheresis has thus been acknowledged to be an accepted form of cellular photoimmunotherapy, the first of its kind approved by the FDA for any cancer (CTCL). New data obtained from the results of numerous recent clinical studies, particularly in the fields of hematology (acute and chronic GVHD), dermatology (systemic sclerosis, NFG), rheumatology (refractory rheumatoid arthritis), gastroenterology (refractory Chron's disease) and solid organ transplant rejection (heart and lung) have provided additional clinical data that will further help better define its role in photomedicine and inter-disciplinary medicine. This is supported by numerous published critical consensus documents (e.g.: EDF consensus guidelines for the use of Extracorporeal Photopheresis, EORTC) both in the US as well as Europe. As the mechanisms of action get further unraveled, it is clear that the potential in 2013 still remains significant for additional significant contributions of this therapy in the areas of photomedicine, photodermatology, photoimmunology, oncology and medicine in general. The unraveling of its mechanisms of action may also help us better understand it effects at an individual level.

IL146

Narrow band UVB phototherapy versus PUVA

Peter Wolf

Department of Dermatology, Medical University of Graz, Graz, Austria

Despite the introduction of new treatment modalities such as biologics, phototherapy continues to keep its prominent place in the dermatologic armamentarium. Phototherapy with oral or topical PUVA and narrow band 311 nm UVB is widely used to effectively treat psoriasis and various other conditions, including eczema, vitiligo, mast cell diseases, graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation, lymphoproliferative disorders such as pityriasis lichenoides and lymphomatoid papulosis, and cutaneous T cell lymphoma. In addition, phototherapy is a prophylactic treatment for certain photodermatoses such as polymorphic light eruption. Choice of treatment and optimal regimes to maximize efficacy and reduce adverse effects is essential and will be discussed in the context of the different types of treatments and phototherapy-responding diseases. The mechanistic action of phototherapy seems to be linked to vitamin-D-inducing, cytotoxic, pro-apoptotic, and/or immunomodulating effects, varying depending on the type of responding disease. For instance in psoriasis, phototherapy suppresses the Th17/IL-23 axis and induces regulatory T cells what relates to efficiency of the treatment. In contrast to other therapies, phototherapy (PUVA more than UVB) is followed by long-lasting remissions (of 6 to 12 months and more in average) after stop of treatment. The reason for this long-lasting efficiency is not well understood and this matter is part of ongoing investigations. The differential safety as well as cancer risk of the different phototherapies will also be covered, in addition to the future of this treatment approaches and potential for new developments, such as digital phototherapy, allowing the ultra-exact, targeted treatment of diseased skin.

IL147

Current state of UVA1 phototherapy

Sally H Ibbotson

Photobiology Unit, University of Dundee, Ninewells Hospital & Medical School, Dundee, United Kingdom

Ultraviolet (UV)A1 (340-400 nm) phototherapy was first introduced over 30 years ago but has not been widely disseminated, with service provision generally limited to specialist centres of photobiology expertise. Reasons for this

include size and expense of equipment and difficulties with installation, the expertise required to deliver the service and the relative lack of a robust evidence-base for efficacy of UVA1 in several diseases. However, UVA1 phototherapy is effective in several inflammatory skin diseases including the fibrosing skin diseases, notably scleroderma, and in atopic eczema. The availability of UVA1 phototherapy for patients with fibrosing skin diseases is invaluable, given the relative lack of effective therapeutic alternatives. For atopic eczema, other therapies (including phototherapies) are available and the optimal use and place of UVA1 in the management of patients with atopic eczema remains to be established. UVA1 phototherapy is generally well tolerated, although the long term photocarcinogenic risk of therapy is not yet fully defined. However, it is likely to be significantly less than that of PUVA. Tertiary dermatology centres should have access to UVA1 phototherapy and this has been highlighted in a recent British Photodermatology Group report [1].

1. Kerr AC, Ferguson J, Attili SK, Beattie PE, Coleman AJ, et al. (2012) Ultraviolet A1 phototherapy: a British Photodermatology Group workshop report. Clinical and Experimental Dermatology 37: 219-226.

IL148

Novel approaches to phototherapy

Olivier Sylvian Reelfs

King's College London, London, United Kingdom

Photochemotherapies such as psoralen plus ultraviolet A (320-400nm) radiation (PUVA) and photodynamic therapy (PDT), in which ultraviolet radiation (UVR: 280-400nm) or visible light is combined with a photosensitizing drug have proven therapeutic effectiveness in a number of non-malignant hyperproliferative skin conditions and various cancers. Nevertheless, all existing photochemotherapies have drawbacks – for example the association of PUVA with the development of skin cancer, and pain that is often associated with PDT treatment of skin lesions. Furthermore, the photosensitizers employed are often not specific for the target tissue. Hence, there is a clinical need to develop alternative approaches that involve lower radiation doses and/or improved selectivity for target cells.

We have been investigating a novel concept - thionucleoside-meditated DNA photosensitisation to low, non toxic doses of UVA radiation. DNA is not a UVA chromophore and skin is about 1000 times less sensitive to UVA than to UVB (280-320nm) radiation. However, we have shown that the incorporation of a thiobase (4-Thiothymidine, S^4TdR) converts DNA of cultured cells into a UVA chromophore and that this results in low dose, largely p53-independent, UVA phototoxicity that is selective for dividing cells. In an organotypic human skin model, UVA penetration is sufficiently robust to kill S^4TdR-photosensitized epidermal cells. Our studies of the mechanism by which S^4TdR/UVA treatment produces its lethal effect have identified several potential contributory factors. These include the photochemical formation of a DNA photoproduct (a thietane) that is analogous to the DNA 6-4Py:Py dimers produced by UVB and UVC radiation, but unlike 6-4Py:Py is not efficiently excised from DNA. These studies also reveal photochemical formation of DNA interstrand crosslinks (ICL). Finally, the low mutagenicity of S^4TdR/UVA, partly attributable to its photoactivation not releasing detectable quantities of reactive oxygen species (ROS), suggests that the combination is a potentially effective and safe therapeutic option.

OC149

The Effects of Narrow-Band UVB (311 nm) Phototherapy on Epidermal Barrier and Differentiation Markers in Polymorphic Light Eruption

Emma J Pond, Catherine A O'Neill, Lesley E Rhodes, Neil K. Gibbs

University of Manchester, Manchester, United Kingdom

The exact aetiology of polymorphic light eruption (PLE) is unknown but is thought to involve a delayed-type IV immunological reaction to unknown endogenous or exogenous 'photoallergens' formed after skin exposure to ultraviolet radiation (UVR). Paradoxically, it has been found that narrow band ultraviolet-B (NBUVB) phototherapy reduces the severity of PLE symptoms, despite acute UVR being a trigger for PLE. How NBUVB phototherapy works in PLE is unknown and the histological effects of phototherapy on PLE skin have not been reported. We therefore investigated epidermal structure and differentiation in the skin of patients with PLE (n=6; age 43-53 yrs, 5 female, 1 male, skin types I-III) before and after NBUVB phototherapy. Patients attended whole-body NBUVB phototherapy (Philips TL-01, peak 311nm) for 3 sessions a week for 5 weeks. Exposures started at 70% of the individual patient's minimal erythemal dose and increased in ~20% increments over the treatment course. Biopsies were taken from PLE skin before and after the phototherapy course. Haematoxylin and eosin was used to stain epidermal structure and immunofluorescence of filaggrin was used to investigate epidermal differentiation. Results showed that the mean overall thickness of the epidermis was significantly reduced, by 19%, after phototherapy ($p<0.001$). In contrast, the stratum corneum (SC) was significantly (27%) thicker ($p<0.05$) after phototherapy. Because of this increase in SC thickness, but reduction of overall epidermal thickness, cell layers were counted. There was no significant difference in numbers of cell layers, but when individual keratinocyte cell thicknesses were measured it was shown that cells were significantly thinner after phototherapy ($p<0.05$). Furthermore, quantification of immunofluorescence of the terminal differentiation marker, filaggrin, showed this to be significantly reduced after phototherapy ($p<0.001$). These results suggest that NBUVB phototherapy has profound effects on epidermal morphology in PLE. The increase in SC thickness noted after phototherapy may compensate for a decrease in epidermal thickness and result in both increased photoprotection and a strengthened barrier against 'photoallergen' ingress.

OC150

Novel light-activated caged iron chelators: targeted prodrugs for iron related-disorders

Benjamin Young, Olivier Reelfs, Asma Aroun, Magnus Hoffmann, Seyedali Miri, Charareh Pourzand, Ian Michael Eggleston

Department of Pharmacy & Pharmacology, University of Bath, Bath, United Kingdom

Iron chelation therapy (ICT) is a well-established approach for the treatment of a variety of conditions that are associated with harmful levels of iron in the body. The involvement of iron in the pathophysiology of cancer, psoriasis, and neurological disorders has stimulated much interest in other applications of ICT, but these require iron sequestration to be targeted to diseased tissues only, thus avoiding depletion of iron in healthy tissue and unacceptable patient side-effects. The concept of light-activated caged iron chelators (CICs) is an ideal solution. CICs are prodrugs that are inactive themselves as iron chelators, but may be simply activated to release a strong iron chelator in a highly spatially-specific and dose-specific fashion after administration, following targeted exposure to light. To further develop this idea [1], we have synthesised a range of chelators of the isonicotinoyl hydrazone family, in which a critical iron-binding phenolic function is blocked ("caged") with the photolabile 2-

nitrophenylethyl (2-NPE) group. These are based on the salicylaldehyde (SIH-like), pyridoxal (PIH-like), and 2-hydroxy-1-naphthaldehyde (NIH-like) templates. Upon exposure to a dose of 250 kJ/m^2 of UVA, the novel CICs cleanly release the parent chelator and a biologically inert fragment. Extent of uncaging was assessed by analytical HPLC, with comparison with authenticated independently synthesised photoproducts. The cytotoxicity of the caged and UVA-irradiated derivatives, and the UVA-generated co-product (nitrosophenylketone) was assessed in the spontaneously immortalised human HaCaT keratinocyte cell line. We have also synthesised CICs in which caging is achieved with groups (6-nitroveratryl, 7-diethylaminocoumarin-4-yl)methyl) that may be more efficiently released at longer wavelengths. Effective release of the parent chelators by irradiation with 365 nm and 420 nm light was again validated by HPLC. This shows the generality of our CIC approach. With suitable caging groups, targeted and wavelength-selective release of chelators can be achieved for a variety of therapeutic applications. In particular, we can tailor CICs to a specific application (e.g. anticancer, photoprotection) by choosing the optimum caging group-chelator combination for maximum tissue penetration of activating radiation and cytotoxic effect of the released chelator.

[1] Reelfs O, Eggleston IM, Pourzand C. Cur. Drug Metabol. 2010, 11:242-249.

OC151

Blue and red LED light promote wound healing in porcine skin

Peter Dungel, Joachim Hartinger, Georg Feichtinger, Heinz Redl, Rainer Mittermayr

Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

Introduction: Treatment of donor sites after meshed skin graft harvesting is important to prevent infections, attenuate pain and regenerate the skin for further harvesting. Low level light therapy (LLLT) can be an efficient means to promote wound healing. Here we aimed to compare the effects of red and blue light from LED on wound healing of skin donor sites in a pig model.05 in

Methods: Eight 0.5mm thick epidermal wounds measuring 5cm × 5cm were made paravertebrally on the back of pigs. Wounds were illuminated post-OP and on day 2 for 10 min with light-emitting diodes (LED) at either 470nm or 630nm with 10mW/cm^2. On day 5 planimetric, histologic and immunohistochemical parameters were analyzed.

Results: In both light treated groups wound healing was enhanced. Red light reduced open wound area by 49%, blue light by 62% ($P<0.01$ vs. untreated control). Gene expression analysis showed down-regulated VEGF-A and VEGF-R2 reaching statistical significance in the blue light group, suggesting that wound healing was already completed. Furthermore, collagen I mRNA was decreased in both light groups, indicating less scar formation.

Conclusion: Our data suggest that LLLT with red and blue light can enhance wound healing processes and may reduce scar formation. LLLT would provide an easily applicable and cost-effective treatment for surface wounds. Lorenz Böhler Fonds 1208.

OC152

Effects of high frequency red light by REPULS® on C2C12 cells

Sidrah Chaudary, Sergiu Dimitrescu, Benedikt Faulhammer, Adelheid Weidinger, Heinz Redl, Peter Dungel

Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

Introduction: There is increasing interest in low-level light therapy (LLLT) to promote wound healing. In-vitro studies have already demonstrated the positive effect of continuous low-level light in the red or infra-red range. Recently Relux Ltd., Austria, introduced REPULS®, a high frequency red light radiation lamp, to the market, which emits cold pulsed (2.5Hz) LED light at 632nm. The aim of this study was to investigate the effects of REPULS®, on C2C12 cells, a myoblast cell line, under normoxic and hypoxic conditions.

Methods: C2C12 cells were illuminated with REPULS® with 200mW/cm^2 every 24-hours for 10 minutes and incubated up to 72h under normoxic conditions. To simulate ischemic conditions, cells were incubated under hypoxic conditions at < 1% oxygen for 3h, and illuminated for 10min before reoxygenation for 6h and 24h. Cell proliferation, metabolic activity and cell viability were determined by BrdU and MTT assays and flow cytometry. ATP production was measured via a luminescence assay and mitochondrial respiration analyzed on an Oroboros-2k oxygraph.

Results: Cellular proliferation was significantly increased by 32% after 24h in the light-treated group compared to the control group. The trend of increased metabolic activity after 24h reached statistically significance after 72h. Flow cytometry data indicated enhanced viability and decreased apoptosis at 6h and 24h post irradiation. ATP levels, determined immediately after irradiation, did not change. C2C12 cells subjected to hypoxia/reoxygenation showed an increase in proliferation at both 6h and 24h timepoints ($P<0.05$) and had a higher metabolic activity after 24h. Respirometric measurements revealed a significantly higher mitochondrial activity in the light-treated group compared to controls.

Conclusions: Our data demonstrate that pulsed red LED light by REPULS® positively influences myoblasts, especially when challenged by hypoxia/reoxygenation. This may contribute to the impressive positive effects of this lamp in wound healing. REPULS® has already been successfully used in orthopaedics, trauma surgery and at rehabilitation centers.

Supported by Relux Ltd, Austria

PL201

Cyclobutane pyrimidine dimers: where they went and what they did there - the path to skin cancer

Douglas E. Brash

Yale School of Medicine, New Haven, CT, United States

The discovery, fifty years ago, that the lethal and mutagenic cyclobutane pyrimidine dimer could be excised from DNA launched a new saga in its illustrious career. As the archetypal target of excision repair, the CPD was used to show that xeroderma pigmentosum, an inherited syndrome predisposing to sun-induced skin cancer, results from a defect in excision repair. Using photolyase as a CPD eraser, lethality and mutations in *E. coli* were shown to stem from errors during excision repair.

The advent of DNA sequencing technology then revealed that mutation hotspots occur at CPD and (6–4) photoproduct hotspots. Both occurred at DNA sequence motifs, enabling hotspots to be predicted from the DNA sequence. CPDs were shown in mammalian cells to be the principal source of the "UV signature" of C T mutations at dipyrimidine sites. A search for the UV mutation signature in skin tumors revealed *p53* and PTCH as genes mutated by sunlight in non-melanoma skin tumors, connecting environmental carcinogenesis to cancer genes. The same mutations were present in skin precancers and as clones in sun-exposed skin. Whole-exome sequencing eventually revealed UV signature mutations in melanoma.

Mutations revealed where CPDs had been, but knockout mice revealed what they had been doing. Mice lacking *p53* were defective in UV-induced apoptosis and this apoptosis protected against skin cancer by eliminating pre-mutant cells. Mice knocked out for transcription-coupled repair were sensitive to inducing P53 and apoptosis, implicating DNA damage as the trigger for this stress response and the transcribed 2% of the genome as the site being monitored. Yet, once an apoptosis-resistant *p53* mutant cell does arise, UV-induced apoptosis favors the mutant and drives its clonal expansion. The same signal also

drives clonal expansion by altering stem-cell fate decisions. From molecule to cell and tissue, the sequelae of CPDs have led – and continue to lead – to a deep understanding of a ubiquitous human carcinogen.

IL202

Sunlight or sardines: establishing seasonal vitamin D sources and status across UK population groups.

Ann R Webb, Richard Kift, Mark D Farrar, Jacqueline L Berry, Lesley E Rhodes

University of Manchester, Manchester, United Kingdom

Public health guidelines in the UK assume that from school age to retirement (ages 4 - 64 years) all vitamin D requirements are met by synthesis of the vitamin in skin after exposure to solar (UV) radiation. The exceptions are “at risk” groups including pregnant and lactating women, and those over 65, for whom 10 µg/day (400 iu) is recommended. Under the age of 4, during rapid growth and with acknowledgement of the vulnerability of delicate skin to sun exposure, an intake of 8.5, falling to 7 µg/day is recommended. These intakes, defined in 1991 and re-assessed and unchanged in 1998, are predicated on avoiding rickets and maintaining a healthy skeleton, with 25 nmol/l (10 ng/ml) of circulating 25-hydroxyviatmin D defined as the lower limit of acceptable vitamin D status. They do not address other potential benefits of vitamin D.

In a series of studies in the Greater Manchester (UK) area we have investigated both the dietary and the sun-induced skin synthesis contributions to vitamin D status, measured by circulating 25-hydroxyvitamin D, as a function of season. Our population groups have included white Caucasian and South Asian (skin type V) adults, white Caucasian and South Asian adolescents, and individuals with photosensitivity disorders. The results must be assessed with respect to changes in our understanding of vitamin D and its relation to both bone health and other disorders, and to societal changes that have influenced our diet and activities over the past 20-30 years.

Despite differences within and between population groups, it was apparent that dietary intake of vitamin D is consistently low year round. Each group has a seasonal cycle in circulating 25(OH)D, albeit of different amplitudes. Summer sunlight increases circulating 25(OH)D, and dietary intake is insufficient to maintain summer levels through the winter, when the sun is too low in the sky to elicit any appreciable vitamin D synthesis in skin. In this respect the basic assumption that vitamin D status is dependent on synthesis in the skin remains correct. The differences between behaviour and vitamin D outcome for various population groups, and the implications for health, will be explored further.

IL203

Spectral dependency of vitamin D photosynthesis and the influence of sunscreen use

Antony Richard Young

King's College London, London, United Kingdom

The action spectra for erythema and pre-vitamin D are similar in the UVB region. There is no evidence that UVA can induce vitamin D synthesis (though this has not been well tested) but UVA makes a varying contribution to solar erythema, dependent on the height of the sun. We assessed the relationship between erythema and vitamin D synthesis with equivalent erythemal doses using three different spectra (UVB content from 1.8 - 25.7%). Participants were exposed to 2 standard erythema doses (SED) of UVR every 3-4 days for a total of 5 exposures over 5% of body surface area. Blood was taken before, during and after the exposures and assessed for 25(OH)D by mass spectrometry. The data showed a UVR dose-response for all spectra, the efficacy of which (~ 4-fold range over different spectra with maximum increase ~ 16 nmol/L) was dependent on the UVB contribution to the erythemal effect (55.5 - 97.8%). These data suggest that vitamin D can be synthesised even when the sun is relatively low and confirm that low UVR doses over small areas of skin can readily induce vitamin D.

Sunscreens designed to inhibit erythema are also likely to inhibit vitamin D production. We conducted a one-week holiday field study in Tenerife in March to assess the degree of vitamin D inhibition when sunscreens are applied at a thickness comparable to sun protection factor (SPF) testing. Participants (n = 40) were given sunscreen which they applied three times daily. The sunscreen group (labelled SPF = 15) was compared with a non-intervention group (n= 22) that brought their own sunscreens and were given no instructions on use. All participants wore a "SunSaver" which electronically records erythemal exposure. Cumulative doses over the week were in the region of 45 SED. Blood samples were taken before and after the study and assessed for 25(OH)D by mass spectrometry. Erythema was recorded daily by reflectance spectroscopy on different body sites. The non-intervention group showed an increase of 28 nmol/L 25(OH)D which was associated with erythema. The sunscreen group showed an increase of 16 nmol/L , but without erythema. A control group (n =17) that remained in central Europe showed a slight decrease in 25(OH)D. These data show that "correct" sunscreen use inhibits erythema and vitamin D synthesis but still allows significant synthesis to occur.

OC204

Seasonal UV exposure, lifestyle, vitamin D3 and UV skin protection

Helmut Franz Piazena[1], Alexandre Moguilevsky[1], Hans Meffert[2], Ralf Uebelhack[2]

[1]Charité- Universitätsmedizin Berlin, Berlin, Germany; [2]Dermatologisches Zentrum Berlin, Berlin, Germany

To measure vitamin D_3 synthesis considering current recommendations of UV skin protection whole-body exposures of healthy, non-adapted volunteers were performed during the end of winter. Volunteers were of different photo-types and of ages between 22 and 60 years and received 15 UV exposures in total. Both spectrum and irradiance were chosen to be similarly with solar exposures at noon-time in summer in central Europe under cloudless skies. First group of volunteers received constant single UV doses (H_{er}) of 30 % of the individual erythemal dose in non-adapted skin (MED_i) three times per week considering a break of one day between two exposures. Second group was also exposed each second day of the week, but by single doses which were increased by 15 % of MED_i after each fifth exposure. In contrast, third group was daily exposed to 30 % of MED_i during the first week, to 45 % during the second week and to 60 % during the last week. A fourth group of volunteers remained unexposed for reference. Whereas 25(OH)D3 serum concentrations ($C_{25(OH)D3}$) of volunteers of the latter group decreased continuously with time, volunteers of the first group showed stagnating concentrations. Increases of 25(OH)D3 concentration in groups 2 and 3 depended significantly on individual age, on the concentration at the beginning of the series and showed decreasing gradients $\Delta C_{25(OH)D3}/\Delta H_{er}$ during the series which could be a hint to both competing effects of adaptation and/or saturation effects. As compared to the results of these series, seasonal gradients $\Delta C_{25(OH)D3}/\Delta H_{er}$ determined for healthy volunteers at Berlin/Germany during spring and summer 2009 and 2010 were up to about five-times smaller for the young adults and up to about two-times smaller for the seniors. This was mainly caused by only episodic or sporadic but frequently excessive UV exposures due to lifestyle, weather conditions and inadequate personal UV protection measures which resulted in high seasonal cumulative UV doses with increasing risk of skin cancer formation but limited seasonal increase of vitamin D_3.

OC205

Sometimes is less more – open problems on optimized recommendations concerning UV-exposure and vitamin D to the public

Peter Manfred Knuschke[1], Andrea Bauer[1], Bodo Lehmann[2], Andrea Pueschel[2], Henriette Rönsch[1]

[1]TU Dresden, Medical Faculty, Dept. of Dermatology, Dresden, Germany; [2]ehem. TU Dresden, Medical Faculty, Dept. of Dermatology, Dresden, Germany

The seasonally change of solar UV exposure in the mid and northern latitudes causes a reduced vitamin D status in the population especially in wintertime. The vitamin D status of more than 75 % of the population is below an optimal range (≥ 30 ng/ml), more than 50 % below a insufficient range (20…30 ng/ml). A lot of work was done in the last years to investigate the relations between UV-exposure and the vitamin-D-status in men. The vitamin D status in its annual course was investigated with respect to the environmental UV-exposure. It was controlled what are the lowest exposures (UV-dose, skin area, body sites) to increase lowered vitamin D level. Investigations were done with artificial UV-exposures in wintertime, to improve the lowered values. For this purpose medical but mostly sunbed UV lamp were used in the studies. The aim of most investigations was to reach the optimal vitamin D status – if possible in short times.

But, on the other hand, there is the problem of the increasing skin cancers risk by the cumulative lifetime UV-dose – e. g. for SCC by a factor 1.8 between outdoor workers and non-outdoor workers.

From our point of view there are several steps necessary to realize a vitamin D status in an optimal range round the year by minimalized additional UV-exposure risk. Recommendations to the public will have to include behaviour information in summertime for times with and without necessary additional protection measures with the aim of an optimization between additional UV-exposure and additional vitamin D production in the skin. From autumn to spring a supplementation should be the preferred way to stabilize the summer vitamin D level in an optimal range. An other way: sunbed use is able to increase a lowered vitamin D status or stabilized the summer level. But the typical sunbed exposures may cumulative increase the skin cancer risk because the annual UV-exposure get levels of outdoor workers. Compared to the efficiency of solar summer spectrum to increase 25OHD in serum the efficiency of sunbed exposure is only 30 % (whole body exposure by 70 % of the individual MED - typical exposure conditions in a sunbed use). But to increase 25OHD in serum: 0.1 MED of sunbed exposure to whole body is effective as 0.7 MED. Or only 0.1 MED to face and hands resulted in 30 % of the 25OHD increase after a typical sunbed exposure (whole body by 0.7 MED)*). Less would be more – for vitamin D production, but without cosmetic tanning effect! Beside this, there are further questions to answer for adequate recommendations to the public. *)German Federal Office for Radiation Protection (BfS): support-N° StSch 4538

OC206

Three sun-simulated UV-B Radiations lead to persistently higher Vitamin D levels and improved mood in healthy young women

Maria Gudrun Biersack[1], Malgorzata Majer[1], Helmut Piazena[2], Tanja Braun[1], Vera Höhne-Zimmer[1], Frank Buttgereit[1], Gerd-R. Burmester[1], Ralf Uebelhack[3], Jacqueline Detert[1]

[1]Department of Rheumatology and Clinical Immunology, Charité- Universitätsmedizin Berlin, Berlin, Germany; [2]Medical Photobiology Group, Charité- Universitätsmedizin Berlin, Berlin, Germany; [3]Department of Psychiatry and Psychotherapy, Charité- Universitätsmedizin Berlin, Berlin, Germany

Objectives: UV radiation is necessary for up to 90% of the physiological Vitamin D (VitD) production and is not sufficiently available in Europe during the winter months. Low levels are often found in patients with depressive disorders. Up to now oral supplementation is the common mode of treatment for VitD deficient patients. The aim of our pilot study was to examine whether a weeklong artificial UV-stimulation can have an effect on VitD status and mood of healthy young women and to detect potential nutritional influences.

Methods: 20 healthy women, aged 20-25 (22,9 ± 1,2), were exposed to three sessions of solar simulated UV-B radiation (on day 1, 3 and 5) during one week in winter. The irradiation dose was increased every session remaining well beyond the minimal erythema dose (MED), defined as 250 J/m². Blood samples for 25(OH)D3 and 1,25(OH)2D3 determination were taken on day 1, 8, 36 and 50. Before (day 1) and after (day 8) the radiation week the participants answered the mood questionnaire "Beck Depression Inventory" (BDI). A standardized nutritional record containing VitD rich food was also completed by the subjects at the beginning of the project to assess the daily VitD intake.

Results: Both VitD metabolites increased considerably after the three UV-sessions: 25(OH)D3 rose from its 54,4 nmol/l baseline level to 68,3 nmol/l on day 8 ($p < 0{,}001$), as did 1,25(OH)2D3 from 130,9 pmol/l to 157,1 pmol/l ($p = 0{,}009$). Moreover, we detected still significantly higher 25(OH)D3 levels than at baseline 4 and 6 weeks after UV-exposure. 1,25(OH)2D3 correlated mildly with the daily VitD intake ($R = 0{,}456$, $p = 0{,}04$), 25(OH)D3 did not. Interestingly, the BDI also showed an improvement of more than 35% ($p = 0{,}003$), which was negatively correlated with the nutritional VitD intake: subjects with low daily intake showed a stronger mood improvement after the radiation week. Likewise, the initially deficient subjects' response to the UV-exposure was with an increase in 25(OH)D3 levels of D 21,8 ± 10,2 nmol/l twice as high as their sufficient colleagues' (D 10,4 ± 6,7) ($R = -0{,}74$, $p < 0{,}001$).

Conclusions: Three suberythemal solar simulated UV-B stimulations increase VitD levels and also considerably improve the mood of healthy young women. After further studies with an expanded spectrum of subjects, this mode of treatment can present a suitable alternative to oral supplementation.

OC207

Population-specific recommendations on sunlight exposure could reduce risk of vitamin D deficiency: results of a dose-response study in UK S. Asians

Mark D Farrar, Ann R Webb, Richard Kift, Marie T Durkin, Donald Allan, Annie Herbert, Jacqueline L Berry, Lesley E Rhodes

University of Manchester, Manchester, United Kingdom

Vitamin D insufficiency and deficiency remain prevalent at northerly latitude, particularly in individuals of South Asian ethnicity. Health agencies advise that low vitamin D status can be avoided through short casual summer sunlight exposures but this advice is geared towards white skinned people. A UV exposure regime simulating this can produce vitamin D sufficiency (25[OH]D ≥20 ng/mL) in 90% of UK white Caucasians but none of the S. Asians attained this level. As skin cancer risk is low in S. Asians, we examined whether higher UV exposure levels could produce vitamin D sufficiency and avoid deficiency (25[OH]D <10 ng/mL).

In a dose-response study, 60 healthy S. Asians (20-60y) received one of 6 UVR exposures ranging from 0.65-3.9 SED, equivalent to 15-90 minutes midday summer sunlight at 53.5°N (Manchester, UK), 3 x weekly for 6 weeks. Exposures were performed in a whole body cabinet (95% 320-400 nm, 5% 290-320 nm), with subjects wearing casual clothes revealing ~35% skin area. Weekly blood samples were taken for serum 25(OH)D analysis. All 51 subjects completing the UV course were vitamin D insufficient at baseline (mean±SD 25[OH]D 6.5±2.8 ng/mL) with 90% deficient (<10 ng/mL). Serum 25(OH)D was significant higher in all dose groups post-course ($P \leq 0.01$) but only 6/51 subjects reached ≥20 ng/mL. The 3.25 SED group attained the highest mean±SD 25(OH)D level (18.0±6.2 ng/mL)

and greatest rise (12.7±7.8 ng/mL), with the highest dose of 3.9 SED failing to produce higher levels. A 25(OH)D level ≥10 ng/mL was achieved by 31/33 (94%) subjects receiving ≥1.95 SED (equivalent to ≥45 minutes unshaded midday sunlight exposure at UK latitude). Initial rise in 25(OH)D appeared linear in all dose groups then started to plateau as the course continued. Consistent with the above findings, a non-linear one-phase association model predicted none of the dose groups to reach a mean 25(OH)D ≥20 ng/mL, but mean 25(OH)D levels in those receiving ≥1.95 SED would plateau at >15 ng/mL.

Current sunlight exposure guidelines are inappropriate for S. Asians living at northerly latitude. Vitamin D status of this population sector could be enhanced by targeted guidance on increased sunlight exposure to achieve a level that avoids deficiency (25[OH]D <10 ng/mL), thus avoiding risk of osteomalacia and rickets, and may assist dietary strategies in attempts to reach ≥20 ng/mL.

IL208

Seasonal Variation in Vitamin D status and Cancer Risk

Frank R. de Gruijl, Jan-Nico Bouwes Bavinck
Leiden Univ. Med. Ctr./LUMC, Leiden, Netherlands

The UV-induced cutaneous production of vitamin D is found to exert a plethora of cellular/physiological effects through its metabolite 1,25dihydroxyvitmin D ligating to its cognate receptor (VDR): e.g., epithelial cells may thus be skewed from proliferation to differentiation and immune responses may be modulated. Such effects could contribute to a lower risk of cancer of various types. Well established is a negative correlation between vitamin D status and colorectal cancer risk.

Studies in rodents have demonstrated that vitamin D can indeed reduce development of intestinal tumors. Our group has extended these findings by showing that moderate physiological dosages of UV radiation suppress the development of intestinal carcinomas in (Apc-mutant) mice on a vitamin D-deficient diet.

In a retrospective cohort study in renal transplant recipients (N =1060) we found a significant increase in risk of internal malignancies with reduced pre-diagnostic vitamin D status in winter (n = 35), but not in summer (n = 38); hazard ratio = 1.3 for each 10 nmol/l drop in circulating 25hydroxyvitamin D in winter ($p = 0.025$). Winter and summer statuses were significantly ($p < 0.001$), but very weakly (R-squared = 0.23), correlated in cancer-free recipients (n = 368), and slightly more strongly in recipients who contracted internal malignancies (R-squared = 0.51, n = 26). Other studies on human cohorts appear to find similar correlations between risk of colon or lung cancers and winter levels of vitamin D, and poor correlations with summer levels.

Based on these results, it would appear more effective to combat a low vitamin D status in wintertime, and not encourage people to seek more sun exposure in summertime to increase their vitamin D statuses.

IL209

Sunlight, Vitamin D and Immunity

Prue H Hart, Shelley Gorman, Royce LX Ng, Naomi M Scott
Telethon Institute for Child Health Research, Subiaco, WA, Australia

Humans obtain 80-90% of their vitamin D through exposure of skin to UV wavelengths and in recent years, multiple immunoregulatory properties of vitamin D have been reported, supported by an association of vitamin D deficiency with poor immune function and increased susceptibility to chronic immune diseases. However, reverse causality, or an association due to lack of outdoors activity, cannot be ruled out. UV irradiation of skin also causes an immunosuppression both locally and systemically and has been implicated in the positive latitude gradients observed for many immune-mediated diseases (e.g. multiple sclerosis). The benefits of vitamin D supplementation for patients with chronic immune diseases, have not been as definitive as hoped and suggest that other mediators that are induced by UV radiation may contribute to, or be responsible for, UV-mediated immunomodulation. Vitamin D levels may provide a proxy measure of UV exposure. Male vitamin D-deficient mice (BALB/c and C57BL/6) do not increase vitamin D levels in response to UV irradiation and provide a model to study vitamin D-independent, UV-induced immunoregulatory mechanisms. We have shown that UV irradiation of skin, by a vitamin D-independent pathway, alters early myeloid progenitors in the bone marrow. Similarly, the time of UV exposure or vitamin D deficiency may be important, with UVR/vitamin D levels during pregnancy associating with the risk of immune-mediated diseases. When pregnant mice were UV-irradiated, dendritic cells differentiating from the bone marrow of progeny were less immunogenic and further suggested an epigenetic effect of UVR, and support for a season of birth effect. Our studies suggest that UV irradiation of skin, via prostaglandin E_2 production, has long lasting effects on bone marrow dendritic cell and macrophage precursors, possibly haemopoietic stem cells, such that differentiated cells have reduced immunogenic properties and contribute to UV radiation-induced systemic immuno-suppression. Mechanistic studies suggest multiple pathways of immunoregulation by UV irradiation of skin and include both vitamin D-dependent and vitamin D-independent processes. Further randomised controlled trials with vitamin D for patients with chronic immune diseases are required.

IL210

VA opsin and Melanopsin (Opn4): Developments and Updates

Stephanie Halford[1], Steven Hughes[1], Susana Pires[1], Irene González-Menéndez[2], Wayne Davies[1], Mark Hankins[1], José García-Fernández[2], Russell Foster[1]

[1]Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom; [2]University of Oviedo, Oviedo, Spain

Birds possess photoreceptors located deep within the brain which regulate both seasonal and circadian responses to photoperiod. These “deep brain photoreceptors” were first identified by Benoit in the 1930’s, who used fine glass rods to stimulate the hypothalamus of ducks with artificial daylengths. Spring-like daylengths stimulated testicular growth whilst short winter photoperiods had no effect upon reproduction. In the 1980’s an action spectrum for photoperiodic induction provided strong evidence that these receptors utilize an opsin/vitamin A based photopigment system. The cellular and molecular identity of these photoreceptors has remained a mystery. Vertebrate ancient (VA) opsin was first described in 1998 and was shown to form a functional photopigment and to be expressed in a small sub-set of retinal horizontal cells and ganglion cells, but not the rods and cones. VA was thought to have a restricted taxonomic distribution, confined to the agnatha and teleost fish. We have recently isolated an orthologue of VA opsin from chicken (cVA) and demonstrated that it can form a functional photopigment *in vitro*. We have also shown that this photopigment is expressed within a population of hypothalamic neurons with extensive projections to the median eminence. On the basis of these results, we suggest that VA opsin-based photoreceptors provide the means of day length detection in birds.

The mammalian retina contains a small population of photosensitive retinal ganglion cells (pRGCs) which utilize the photopigment *Opn4*. We have shown that three discrete classes of light-induced Ca^{2+} change can be identified in the pRGCs: sustained; transient and repetitive. The basis for these different responses remains unclear and it is unknown whether the various pRGCs project differentially to the retinorecipient regions of the brain. Recent work by our group has shown that two splice variants are transcribed from the mouse *Opn4* locus. Both isoforms (*Opn4L* and *Opn4S*), which only differ in the lengths of their C-terminal tails, form a fully functional photopigment when

expressed *in vitro*. Isoform specific antibodies have identified discrete populations of retinal ganglion cells, some express both Opn4L and Opn4S and others only Opn4L. Whilst we have not yet identified the functional significance of the C-terminal splice variants of melanopsin, it seems likely that they are associated with the diversity in pRGC light responses.

IL211

Control of Gi/o signaling, neuronal activity and behavior by light activated GPCRs

Stefan Herlitze

Ruhr-University Bochum, Bochum, Germany

The tractability of light activated receptors makes them attractive tools by which to study the brain. They allow for the non-invasive and specific control of neuronal signaling and could allow for the study of receptor pathways that occur faster than the rate of diffusion. Furthermore, with the aid of implantable light devices light activated receptors have the potential for use in live animals and later in humans to control and cure GPCR pathways involved in diseases. We demonstrate here the use of vertebrate rhodopsin to control ion channel modulation, spinal cord, cerebellar and serotonergic signaling via activation of the pertussis toxin sensitive Gi/o pathway by light.

IL212

Unusual opsin photopigments as optogenetic tools to control and manipulate cellular and behavioural activities

Carlo Musio[1], Silvia Caponi[1], Lavinia Liguori[1,2]

[1]CNR Istituto di Biofisica - UOS Trento, Trento, Italy; [2]TIMC Lab., Equipe SyNaBi, UMR CNRS 5525, La Tronche, Grenoble, France

The ”World of the Opsins”, as we knew it until about a decade ago, has been revolutionized in few years by the discovery of novel opsins within non-rod non-cone and extra-retinal photoreceptors, and of new microbial rhodopsins that share the function of both photoreceptor and membrane channel or pump. The former are ubiquitous among Metazoa, from cnidarians to humans, and serve non-visual photoreception (i.e. non image-forming or circadian vision). The latter have been discovered in Archea and microalgae only and control functions such as phototaxis, energy storage, development, and retinal biosynthesis. Since 2005 a new opsin-based technology, named optogenetics, was developing to control and manipulate neural activity at various level of complexity (from single cell to cellular via neural circuits) with an extremely high spatio-temporal resolution. Optogenetics mainly employs microbial opsins as actuators to transduce the photic signal (corresponding to the specific wavelength exciting the chromophore) into physiological signals to make cellular function controllable. Amongst others, channelrhodopsins, ChRs, (discovered by Nagel in 2002 and 2003 in the unicellular alga *Chlamydomonas*) are directly light-gated ion channel becoming permeable to cations upon blue light stimulation and they appear the most versatile to be expressed in several cell model systems. In ChRs-expressing cells, a 460-480 nm light stimulation is able to depolarize the cell membrane producing an excitation of the system (opposite action is produced by halorhodopsin). Modifying the components of the ChR structure-function relationships (e.g., gene and protein sequences, photocurrent, photocycle), the protein can be mutated and tailored with respect to absorption, substrate specificity, kinetics, and improved expression in host systems. Then ChRs are implemented for optogenetic applications *in vivo* to evaluate their viability as genetically-encoded photoswitches able to perturb specific cellular and/or crosstalk activities. Very recently also metazoan opsins, such as the non-visual opsin melanopsin of mammals and rhodopsin of the box-jellyfish *Carybdea*, have been demonstrated useful tools for the optogentic control of G protein-coupled receptors (GPCRs) signaling. Opsin-like photopigments have been proven to be suitable optogenetic tools to activate motor functions, to regulate heart function, and to restore vision. Future challenges will be the characterization of new optogenetic tools and their combination to achieve a multi-wavelength control and tuning of biological processes and networks.

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IL213

Archaebacterial halorhodopsin restores vision in the retina

Jens Duebel

Vision Institute Paris, Department of Visual Information Processing, Paris, France

The insertion of light sensitive microbial opsins into retinal neurons is a promising approach to restore vision in retinal degenerative diseases, such as retinitis pigmentosa. In this disease, rod photoreceptors die early, whereas light-insensitive, morphologically altered cone photoreceptors persist longer. To restore vision in mouse models of retinitis pigmentosa, we genetically targeted a light-activated chloride pump (*Natronomonas pharaonis* halorhodopsin, NpHR) to light-insensitive cone photoreceptors by means of adeno-associated viruses. Treated photoreceptors are able to drive sophisticated retinal circuit functions (e.g. lateral inhibition, directional selectivity), activate neuronal networks in the visual cortex, and mediate visually guided behaviors. In human ex vivo retinas, halorhodopsin can reactivate light-insensitive photoreceptors. Blind patients with persisting cones for potential halorhodopsin-based therapy have already been identified. Currently, we are testing halorhodopsin function in the monkey retina in order to translate this optogenetic therapy into clinical trials.

IL214

Photosensitisers – Opportunities in Infection Control

Mark Wainwright

Liverpool John Moores University, Liverpool, United Kingdom

While the rates of hospital-acquired infections due to headline Gram-positive pathogens such as MRSA and *Clostridium difficile* have shown decreases in the past year, in most cases these result from changes in ward cleaning protocols, hand-washing regulation etc. Conversely, infections due to Gram-negative bacteria have increased. In neither case has any advance in conventional chemotherapy been made and without this the prospects for infection control in healthcare remain bleak, unless alternative approaches are taken up.

The photodynamic approach offers much in terms of local disinfection, whether in bacterial, viral, fungal or protozoal disease. Given an essentially non-toxic photosensitiser and a directable light source most foci of disease should be susceptible, and there are good examples of this in surface treatments for herpes simplex and onychomycosis, as well as internal treatments for tuberculosis and oral/nasal decolonisation. In addition, the application of photosensitisers to materials for healthcare use offers photodisinfection/photosterilisation capability in textiles and plastics used in areas such as clothing, woundcare and prosthetics.

Novel photosensitisers, representing improvements on their lead compounds and designed solely for anti-infective end use, continue to be reported. However, the legislative requirements to allow their progress to clinical reality represent, in the majority of cases, too great a financial risk for the process to be attempted.

In terms of the scientific basis, there is now clearly a considerable body of work underpinning the photodynamic approach to infection in a range of applications. What is required for progress is both the sustained effort of those actually involved in clinical work, in addition to the increased involvement of health provision agencies and the pharmaceutical industry.

IL215

Clinical application of antimicrobial photodynamic therapy to chronic disease states

Nicolas G. Loebel[1,2], Roger Andersen[1,2], Merrill A. Biel[1], Chet Sievert[1], Carolyn Cross[1,2]

[1]Sinuwave Inc., Bothell, WA, United States; [2]Ondine Biomedical Inc., Vancouver, BC, Canada

The cost associated with chronic diseases such as chronic rhinosinusitis, periodontal diseases, multi-drug resistant staphylococcal colonisations and biofilm contamination of medical devices such as endotracheal tubes is staggering with respect to morbidity, lost work productivity and direct and indirect healthcare costs. The diseases are characterized by acute exacerbation phases followed by periods of quiescence, a recipe for generation of antibiotic resistance when oral systemic therapy follows the recurrent course of the disease. A resonant host inflammatory response is prevalent, paradoxically partially responsible for maintenance of the chronic condition by generation of local tissue damage, host metalloproteinase upregulation and generation and circulation of acute phase inflammatory proteins such as CRP. The management of these chronic diseases is a challenge given the role of underlying inflammation, the prevalence of difficult-to-treat biofilm pathogen reservoirs and increasing antimicrobial resistance.

Topical antimicrobial photodynamic therapy (aPDT) has been demonstrated to provide high levels of efficacy against a wide range of clinically-relevant human pathogens in biofilm forms, while preserving the structure and function of underlying tissues. The technique does not upregulate antibiotic resistance. A range of photoantimicrobials exist, but the phenothiazinium class provides certain advantages relevant to the treatment of chronic, indolent, human diseases. Clinical studies deploying aPDT against chronic human diseases such as periodontal disease, chronic rhinosinusitis and ventilator-associated pneumonia will be summarized with a view to demonstrating the unique safety profile and the dual mode of action of aPDT against the biofilm organisms themselves as well as the local inflammatory response. Reference will also be made to recent studies by others demonstrating induction of host immunity at the treatment site when phenothiazinium photoantimicrobials are used.

IL216

Porphyrin-photosensitised processes for improvement of water quality through microbicidal and larvicidal action

Giulio Jori

University of Padova, Padova, Italy

The well-known efficiency of cationic porphyrins and their tetrapyrrolic analogues to act as microbicidal and larvicidal factors, once they are electronically excited by irradiation with visible light wavelengths or even sunlight, can be exploited for addressing important problems connected with improvement of the quality of environment.

Thus, laboratory studies have shown that the immobilization of selected meso-substituted porphyrins on solid supports, which can be readily swollen or solvated by water (e.g., Sephadex or cellulose resin), allows the photosensitized killing of a variety of pathogens (e.g., bacteria, fungi, parasitic protozoa in the vegetative or cystic stage) which often proliferate in aqueous ecosystems. This approach has been successfully applied for the photosensitized disinfection of waters from fish-farming systems, in order to protect adult fish and/or fish eggs from infectious diseases, which are responsible for heavy losses in the population of farmed fish, as well as for the spreading of the diseases to other constituents of the aqueous media. The irradiation of polluted waters is performed in a specifically developed chamber, from which the disinfected water flows into the aquaculture tanks; as a consequence, no direct contact between the photoexcited porphyrin or the photogenerated reactive cytotoxic species and the fish takes place. This prevents any toxic or phototoxic effect on the fish and allows a prompt recovery of the porphyrin after irradiation, substantially lowering the cost of the overall operation.

Analogously, stable complexes between powdered selected animal food (AF) and porphyrins have been prepared. These exhibit a sufficiently long floatability (up to one week) in aqueous media, where mosquitoes such as *Anopheles*, *Aedes*, *Culex* (which are vectors of epidemic diseases including malaria, dengue fever, etc.), deposit their larvae. The AF-porphyrin adduct is also very palatable for larvae, so that overnight incubation of the larvae with millimolar concentrations of the adduct causes the ingestion of sufficient amounts of photosensitiser to induce a fast extensive killing of the larvae upon sunrise. This approach can be used for controlling the population of potentially noxious insects and preventing a number of water-borne diseases.

IL217

Photosensitized water treatment through solar reactors - Activated Ru(II) and C60 derivatives

David García-Fresnadillo

Dpt. Organic Chemistry, Faculty of Chemistry, Complutense University, Madrid, Spain

Photosensitized water treatment using sunlight and dissolved oxygen as natural resources may be considered as one of the emerging green technologies for water disinfection and decontamination. A solar reactor for water treatment requires efficient sunlight collectors, optimized rheology and appropriate photosensitizing materials. Wise selection of photostable dyes and inert polymer supports is a key issue in the development of novel and ecologically-friendly water disinfection techniques.

Ru(II) complexes with polyazaheterocyclic ligands (2,2'-bipyridine or 1,10-phenanthroline derivatives) and carbon nanostructures such as those related to C60-fullerene display good singlet oxygen production quantum yields ($0.4 < \Phi_\Delta < 1$) and high photostability. Moreover, they can be easily immobilized, at a sufficiently high concentration, in a variety of inert polymer supports such as nylon, cellulose, perfluorinated polymers, silicone or glass, via encapsulation, adsorption, electrostatic interaction or covalent bonding.

Preparation and photophysical characterization of a series of singlet oxygen photosensitizing materials based on Ru(II) complexes and C60 structures is discussed in terms of i) sensitizer type and loading in the polymer support, ii) efficiency of triplet state quenching by molecular oxygen (Po_2^T), iii) singlet oxygen production (τ_Δ, Φ_Δ), and iv) photoinactivation of *E. coli* and *E. faecalis* bacteria ($10^2 - 10^4$ CFU mL^{-1}).

The sensitizing material that showed better bacteria photoinactivation properties under sunlight was scaled up and tested in solar reactor prototypes comprising compound parabolic collectors (CPC) with two different configurations (coaxial and fin type). Operational parameters such as solar collector configuration, water flow rate, temperature, accumulated radiation and operation time are discussed. Regarding the durability of the photosensitizing material, dye reloading of the aged material is possible, extending the operational lifetime of the photocatalyst. The reactor prototypes developed can be applied to point-of-use $^1O_2$-mediated solar water disinfection.

IL218

Photoinactivation of multidrug-resistant bacteria in hospital wastewaters

Maria A. F. Faustino[1], Joana Almeida[2], João P. C. Tomé[1], Augusto C. Tomé[1], Maria Graça P. M. S. Neves[1], José A. S. Cavaleiro[1], Ângela Cunha[2], Adelaide Almeida[2]

[1]Department of Chemistry & QOPNA, University of Aveiro, Aveiro, Portugal; [2]Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal

One of the main environmental concerns related with hospital effluents is their discharge without preliminary treatment,

together with urban effluents. The traditional methods used to reduce the high content of enteric pathogens, including multidrug resistant bacteria and viruses, of residual waters are based in disinfection processes that are expensive, unsafe and not always effective. Consequently, new technologies are needed, especially for effluents treatment including hospital residual waters. The antimicrobial photodynamic inactivation (aPDI) may represent a potential alternative to traditional methods. The inactivation of clinical multidrug-resistant (MDR) bacteria in hospital wastewaters by PDI could allow achieving important environmental gains and in terms of public heath. The main goal of present work was to assess the inactivation of four clinical multidrug-resistant (MDR) bacteria in PBS and in hospital wastewaters in order to develop a protocol to be used as a basis for PDI field implementation in the future.

Acknowledgments

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OC219

Antimicrobial properties of photoactivated ZnO nanoparticles

Zivile Luksiene

Vilnius university, Vilnius, Lithuania

It is well documented that most of the harmful and pathogenic microorganisms are able to develop high resistance to many conventional chemical fungicides and disinfectants. During the past decade the emphasis in postharvest fruit protection has shifted from using chemicals to various alternative techniques including physical antimicrobials. To this end, the development of novel nano-sized antifungal and antibacterial agents seems promising.

The antibacterial activity of ZnO nanoparticles (ZnO NPs) in combination with visible light (λ = 400 nm) against *Escherichia coli* O157:H7, *Listeria monocytogenes* $ATC_{L3}C$ 7644 and *Botrytis cinerea* was investigated. The antimicrobial properties of photoactivated ZnO NPs were tested also on wheat seeds. Data indicate that photoactivated ZnO NPs (200 nm) have strong bactericidal activity against both bacteria, achieving more than 7 log reductions in bacterial counts. Scanning electron microscopy (SEM) images of treated bacteria indicated cell wall disintegration and cell lysis. Results obtained on examinations of antifungal activity of ZnO NPs reveal that remarkable photoinactivation (33-58%) of *B. cinerea* was observed at concentration $1\times10^{-3} – 5\times10^{-3}$ mol l^{-1} and incubation time 24h. SEM data analysis confirmed susbstantial morphological changes in microfungus. Photoactivated ZnO NPs can reduce the microbial contamination of seeds by 70% without harmful effects on their germination.

Such ZnO NPs properties obviously could be used for the development of effective fungicides in agriculture or innovative physical antibacterial agents, so important in medicine and food microbial control.

OC220

Metallophthalocyanines for antimicrobial photodynamic therapy: An overview of our experience

Vanya N. Mantareva[1], Ivan Angelov[1], Dieter Wohrle[2], Ekaterina Borisova[3], Vesselin Kussovski[3]

[1]*Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria;* [2]*Bremen University, Bremen, Germany;* [3]*Institute of Electronics, Sofia, Bulgaria*

Metal phthalocyanine complexes with different charges, hydrophobicity and metal ions were synthesized and studied for antimicrobial photodynamic therapy of pathogenic bacterial and fungal model strains. Ten positively charged complexes with the metals Zn(II), Al(III), Ga(III)), In(III), Si(IV) and Ge(IV) in the center of the ligand and substituents at the ligand bearing four or eight N-alkylpyridyloxy groups were prepared. In addition, a negatively charged Zn(II)-phthalocyanine with four sulfophenoxy-groups was synthesized. The absorption spectra showed low intensity of the Soret band in the UV part of the spectrum and the intense Q band in the red to far red region (λ = 671 - 697 nm). The fluorescence was determined with quantum yields between 0.1 - 0.33 and life-times 2.8 - 4.9 ns in dependence of the kind of metal ion and the substituents. In organic solvents all complexes exist in a monomeric state but in aqueous solution they show aggregation with exception of Ga(III) phthalocyanines. The singlet oxygen quantum yields were evaluated in dependence on the metals, substituents and the media with values between 0.16 - 0.68. The cationic metal phthalocyanines were taken up by pathogenic cells in a higher amount as compared to the anionic complex. Three of the studied phthalocyanines namely tetra-N-methylpyridyloxy-phthalocyanine Zn(II) and tetra- and octa-N-methylpyridyloxy-Ga(III) phthalocyanines showed a high photodynamic efficacy towards most of the studied microorganisms in suspensions.

OC221

Fluorescent sensor for bacterial recognition

Rehab Amin, Soad Elfeky

NILES ,Cairo University, Cairo, Egypt

Boronic acid-based fluorescent sensor is one of the non enzymatic methods used for the recognition of saccharides. Since bacterial membrane has polysaccharides with diol groups, boronic acids probe could be applied for rapid bacterial recognition. *Escherichia coli* (XL-1 blue) were recognized by applying (3-(5-(dimethylamino) naphthalene-1- sulfonamide) phenyl) boronic acid (DNSBA) as a sensor and the fluorescence recorded by fluorometer micro-plate reader. Results showed that, fluorescence records of DNSBA increase in a dose dependent manner upon increasing the bacterial cell number. Moreover, the increase in the number of bacterial cells induces a shift in the spectra due to the formation of the anionic form of boronic acid complex. Therefore, DNSBA is an efficient sensor for monitoring bacterial cells.

IL222

Emerging applications for PDT

Colin A. Morton

Department of Dermatology, Stirling Community Hospital, Stirling, United Kingdom

Topical PDT is a well-established therapy option for actinic keratosis, squamous cell carcinoma in-situ as well as superficial and thin nodular basal cell carcinomas in immunocompetent patients, with potential for use in field dysplasia and actinic cheilitis. Recent European consensus guidelines reviewed the evidence supporting additional indications for PDT in Dermatology. Despite reduced efficacy, PDT can be used to treat non-melanoma skin cancers in organ transplant recipients, with the additional potential to delay/prevent new lesions although direct evidence of prevention of invasive squamous cell carcinoma remains limited and the cost-effectiveness of a preventive cyclical therapy in the immunosuppressed requires further study. PDT has also been studied in patch/plaque-stage cutaneous T-cell lymphoma, with efficacy more likely in unilesional disease and can be useful in an adjuvant role in extra-mammary Paget's disease.

Accumulating evidence supports the use of PDT in acne and several other inflammatory/infective dermatoses including cutaneous leishmaniasis, infected and diabetic leg ulcers, although protocols are still to be refined. Despite proven efficacy, PDT is little used in viral/genital warts, where pain during treatment can be intense. Improvement in appearances of scars has been observed by several authors and requires further assessment. PDT is a therapeutic option for photorejuvenation,

improving fine wrinkles, mottled hyperpigmentation, roughness and sallowness. Topical PDT is therefore emerging as a useful therapy platform in Dermatology, with potential to treat and prevent skin cancer as well as promote photorejuvenation.

Morton, C.A., Szeimies, R.-M., Sidoroff, A. and Braathen, L.R. (2012), European guidelines for topical photodynamic therapy part 2: emerging indications – field cancerization, photorejuvenation and inflammatory/infective dermatoses. Journal of the European Academy of Dermatology and Venereology. doi: 10.1111/jdv.12026

IL223

PDT of skin infections (wounds and burns) in animal models

Michael R Hamblin, Tianhong Dai, Daniela Vecchio, Yunsong Zhang

Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, United States

Alternative antimicrobial strategies are urgently required to combat the relentless worldwide increase in antibiotic resistance in virulent bacteria and other microbial pathogens. Photodynamic therapy (PDT) can be used to selectively kill microbial cells in wounds and burns without damaging the host tissue. Not only are multi-resistant bacteria equally susceptible, but PDT itself does not induce resistance. Antimicrobial PDT is rapid-action, broad-spectrum and is effective in damaged tissue with compromised circulation where antibiotics cannot reach. We have studied the effectiveness of antimicrobial PDT in small animal models of localized infections using stably-engineered bioluminescent microbial cells that allow real time, non-invasive optical imaging of the progress and spread of infection. We have designed and studied innovative highly-effective antimicrobial photosensitizers with cationic functionality to improve microbial targeting. We have shown the efficacy of PDT in wounds (excisions and abrasions) and third-degree burns in mice infected with *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Proteus mirabilis*, methicillin-resistant *Staphylococcus aureus* and *Candida alibicans*. In some circumstances mice can be saved from death due to sepsis, and in other cases the wound healing is significantly improved by PDT.

IL224

Protocol developments in topical PDT: Science and outcomes

Carola Berking

Department of Dermatology and Allergology, University of Munich (LMU), Munich, Germany

Since the introduction of MAL-PDT using a 16% methyl aminolevulinate (MAL) cream in combination with red light more than 10 years ago, topical PDT has become a well-established and widely recognized first-line method for the treatment of non-melanoma skin cancer. More recently, new application forms of the photosensitizing agent 5-aminolevulinic acid (ALA) have proved to be at least as effective MAL and got officially approved for the treatment of actinic keratoses by the European Medicines Agency. A self-adhesive ALA patch, which contains 8 mg of ALA in a crystalline formulation, can be applied without the need of an additional occlusive dressing and has an incubation time of 4 h. In two phase III trials, the efficacy rates on a lesion basis were 82-89% after 3 months and 63-79% after 12 months. Another new formulation is a 10% ALA nanoemulsion (BF-200 ALA), which is applied for 3 h prior to red light illumination. The patient complete clearance rates ranged from 64-96% after 3 months and 47-69% after 12 months depending on the red light source used. The lesion complete clearance rates were 81-90% three months after the last PDT (1 or 2 cycles).

Another more recent development is the so-called daylight PDT, which uses natural sun exposure instead of red light devices with a modified protocol of MAL application. The main advantage of daylight PDT is the nearly pain-free and more convenient procedure.

A new form of illumination represents the subluminescent PDT (SL-PDT) using a focused, high intensity red light through a fibre optic light guide inserted through a canula under the area to be treated. We have gathered experience with recalcitrant viral warts at the feet with red light illumination from both top and beneath the lesion after application of MAL and the data will be presented.

IL225

Physical techniques to enhance uptake of prodrug into skin tumours

Merete Haedersdal

Bispebjerg University Hospital, Copenhagen, Denmark

Topical PDT is a mainstream treatment for premalignant lesions and selected cases of NMSC. In Europe, PDT is traditionally delivered with MAL and red LED light, which is highly effective for thin dysplastic lesions. However, PDT is less effective in the treatment of thicker lesions. Thus, there is a need to look for alternative and more intensive ways of delivering PDT.

New available physical techniques are available with fractional lasers and microneedling to increase the uptake of prodrug into skin tumors. Currently available data on microneedling- and fractional laser-assisted PDT is discussed and the topic will be covered from basics to clinics, focusing on protocols for dysplastic lesions. New clinical data will be presented on actinic keratoses in immunocompetent and immunosuppressed patients.

OC226

Psychological influence on acute pain during topical PDT for non-melanoma skin cancer

Janet WH Ling, Simon P Kennedy, George Evangelou, Donal G Fortune, Lesley E Rhodes, Helen L Richards

Photobiology Unit, Dermatology Centre and Department of Clinical Psychology, University of Manchester and Salford Royal Hospital, Manchester, United Kingdom

Psychological influences are important in the experience of chronic pain, but less well understood in acute pain. The acute skin pain induced by topical photodynamic therapy (PDT) of non melanoma skin cancer (NMSC) provides a situation for its further study, and findings could indicate psychological strategies for management of acute pain induced by PDT. Thus, our objective was to identify how psychological parameters may influence acute PDT-induced pain.

This study was performed in 60 consecutive NMSC patients referred for topical methyl aminolevulinate-PDT. A cross-sectional, questionnaire based design was employed, with patient completion of the Selves Questionnaire (SQ), Pennebaker Inventory of Limbic Languidness (PILL), Short form of McGill Pain Questionnaire (SF-MPQ), and the Hospital Anxiety and Depression Scale (HADS).

Among the 60 white Caucasian participants, 27 (45%) were male and 33 (55%) female, mean ages 66.5 (SD 11.2) and 60.4 (SD 13.4), respectively. All had NMSC (actinic keratosis, Bowen's disease or superficial basal cell carcinoma). The mean SF-MPQ score for PDT-induced pain was 8.19 (SD 4.98), comparing with their mean VAS score of 3.47 (SD 3.0). The SQ had inter-rater reliability of 0.91 - 0.99 for the self-discrepancies ideal: own, ought: own, ideal: other and ought: other. Self-discrepancies correlated with psychological distress: ideal: other discrepancy with depression ($r = 0.33$, $p<0.01$) and ought: other discrepancy with anxiety ($r = 0.30$, $p<0.05$). Notably, SF-MPQ correlated with ought: other self discrepancy ($r = 0.30$, $p< 0.05$), and ought: other self-discrepancy significantly contributed to PDT-induced pain ($F = 4.27$, $p<0.05$). While pre-PDT pain was usually absent, with median PII score of 0 (range 0-3), there was an association between pre-PDT PPII and SF-MPQ ($F = 3.39$, $p<0.05$).

In conclusion, this study finds that ought: other self-discrepancy, acting through promotion of psychological distress, promotes acute pain in topical PDT. Further studies are warranted to examine how psychological assessment and modification may assist in management of PDT-induced pain in patients requiring multiple treatments. Furthermore, greater attention is warranted to psychological factors in other situations where acute pain is anticipated.

OC227

The benefits and limitations of daylight PDT as predicted by Monte Carlo modelling

Catherine Louise Campbell[1], Kenneth Wood[1], Ronan Valentine[2], Tom Brown[1], Harry Moseley[2]

[1]University of St Andrews, St Andrews, United Kingdom; [2]The Photobiology Unit, University of Dundee, Dundee, United Kingdom

It is important to investigate the light distribution and photon interactions below the surface of the skin during photodynamic therapy to further understand and optimized the treatment procedure. Monte Carlo radiative transfer (MCRT) modelling solves the radiation transfer equation using the probabilistic nature of photon interactions and can be used to trace the paths of multiple photons from a simulated light source through a simulated tissue structure. The method that we used can directly give information about photon interactions that is otherwise impossible to obtain using other methods (e.g. penetrations depth and energy absorption).

By applying MCRT modelling we aim to theoretically investigate the efficiency of different treatment methods and light sources. We will present preliminary results for modelling of different three dimensional tumour structures for different treatment techniques. Including different light sources for different treatment conditions. Based on previously established toxic thresholds for the photodynamic dose, our models allow us to determine the depth at which necrosis occurs and the overall treatment time. We also simulate fluence rate and photobleaching affects to further investigate PDT. Preliminary results support the usage and further development of daylight-activated PDT.

We have performed an analysis of the relative efficacy of a standard LED light source (Aktilite) emitting at 630 nm and sunlight. Porphyrin absorption of sunlight is far greater than from the Aktilite because of the different spectral distribution. Against this, is the fact that sunlight emits much less light at 630 nm and so will have less penetration than the Aktilite.

According to our model, daylight PDT can induce a phototoxic dose at a depth of about 2 mm. Assuming uniform initial porphyrin concentration and wavelength dependent optical properties, the model shows that effective light doses at different depths in the tumour can be achieved with treatment times less than an hour using sunlight as the source spectrum in the model.

IL228

Accumulating evidence for daylight-mediated PDT in actinic keratosis

Hans Christian Olsen Wulf

Bispebjerg University Hospital, Copenhagen, Denmark

Standard PDT-treatment of AK is performed with ALA or MAL, and illumination from red diodes. The treatment is time consuming, mostly because the patient must return to the clinic for illumination after three hours. The main problem for the patient is pain during the illumination which can be severe, about 7 on a pain measuring scale from 0-10, and especially severe when treating face and scalp.

The introduction of daylight PDT simplifies the procedure as only pretreatment and application of the MAL are performed in the clinic. The patient can return to his home and stay outdoors for two hours half an hour after application of the cream. Studies have shown that one treatment of thin AK's will result in nearly 80% clearance. This has been found in several studies performed in Scandinavia and outdoor treatment can be used from around April until November in the northern European countries. By illuminating with daylight during PPIX-formation pain score is dropping to about 1,5 on the pain measuring scale and is thus negligible. Planning of treatment is easy and the capacity of the clinic improves.

New studies in Australia have proved the beneficial effect described above with an even better cure rate of about 90% after one treatment. In the southern part of Europe and in the subtropics treatment can be performed outside all year around, but it has then to be combined with the use of sunscreen. In these locations exposure in the shade is sufficient for treatment efficacy.

IL229

Melanocytes, melanoma and ultraviolet radiation: an overview

Dorothy Bennett

St George's, University of London, London, United Kingdom

Melanocytes are the cells that make the pigment melanin in mammalian skin, hair and eyes, and in feathers and scales of other species. In normal humans the epidermis contains melanin, through transfer of melanosomes (pigment organelles) from melanocytes to the basal keratinocytes. The melanin can be either eumelanin (black to brown) or sulphur-containing pheomelanin (red to yellow). Humans vary widely in the amounts and proportions of these. These quantities depend largely on genetic makeup and determine the colour of hair and eyes as well as skin: socially as well as biologically important. Skin types can be defined according to colour and the response to sunlight/ultraviolet radiation (UVR). Some people are unable to tan, and this associates with light skin and red hair, and notably with mutations at the *MC1R* "red hair" locus, encoding the melanocortin 1 receptor. Various other genetic loci affecting skin colour also associate with melanoma risk.

Melanin has value in protecting against sunburn, skin cancer and dermal photoageing, especially in naturally dark skin. UVR also has a health benefit, in inducing vitamin D synthesis. However there are negative aspects, especially for paler people. The tanning response is now known to involve UVR-induced DNA damage. This induces *p53* signalling, which in keratinocytes promotes secretion of growth factors that stimulate melanocytes to make and transfer melanin. Thus a tan is an indicator of DNA damage in the basal epidermis. Moreover UVR is the accepted principal mutagen in both cutaneous melanoma and non-melanoma skin cancer, with the UVA waveband now implicated in melanoma as well as UVB. Accordingly exposure to all UVR – while not totally avoided – should be treated with caution.

IL230

How does USF1 orchestrate the UV-response

Marie-Dominique Galibert

CNRS-UMR6290 IGDR - University of Rennes, Rennes, France

Cancer incidence increases worldwide and fatal outcome remains elevated despite advances in targeted therapies and early diagnosis. In 2030, the number of patient living with a cancer is expected to reach 75 millions. Knowing that 70% of the cancers are explained by lifestyle and behaviors it is extremely important to better identify and characterize the environmental risk factors to established protective guidelines and develop further new therapies.

Solar UV radiations and sun-tanning beds are clearly responsible for the development of skin cancers the world's most common cancers, however the exact mechanisms remain to be elucidate.

Investigating the molecular impact of UV radiation, we identified the p38 stress-responsive kinase and the Upstream Stimulating Factor 1 (USF1) transcription factor as key actors of the UV-response. We could show that USF1 elicits a skin protection

program against UV-induced DNA damage by regulating the expression of genes implicated in complementary pathways: the UV-induced tanning response and the recognition of DNA-photo lesions. In an attempt to further decipher the biological impact of the loss of the p38-USF1 activation pathway, we examined by which means is controlled the proliferation rate of USF1 KO cells and mice epidermis, challenged by UV-radiation. This led to explore further the interplay and hierarchy between USF1 and the *p53* tumor suppressor pathway.

Taken together our findings revealed a new role of USF1 as a key actor of *p53* stabilization, suggesting that impairment of the USF1 pathway may promote genome instability in response to environmental insults.

Baron, Corre, et al., *PloS Genet.* 2012 ; Mouchet et al., *PLoS ONE*, 2010 ; Corre et al., *J. Biol. Chem.*, 2009 ; Corre et al., *J. Invest. Dermatol.*, 2006 ; Galibert MD, *EMBO J.* 2001.

IL231

Induction of beta-catenin in response to UV radiation: cellular and physiological consequences

Valerie Petit, Irina Berlin, Juliette Bertrand, Lionel Larue
Institut Curie, Orsay, France

Excessive exposure to ultraviolet (UV) rays may lead to the transformation of melanocytes into melanoma, the most aggressive type of skin cancer. For long time, it has been thought that UV were the principal driver for melanoma initiation, however recent deep-sequencing of melanoma samples show that the incidence of UV-induced mutations are much less than expected. On the other hand, one third of melanoma cell lines and primary melanomas have been shown to overproduce nuclear beta-catenin, suggesting a role for beta-catenin in melanoma genesis. We therefore decided to evaluate the molecular mechanisms associated with UV-induced melanocytes transformation and investigate the effect of UV irradiation on beta-catenin signalling in melanocytes. As expected, the irradiation of melanocyte or melanoma cells by UVB induces a rapid phosphorylation of p38 kinase. This kinase is able to phosphorylate and inactivate GSK3, leading to an increase of the stabilized beta-catenin pool. As a consequence, beta-catenin translocates into the nucleus in response to UV irradiation. These results were confirmed in vivo; mouse pups were UVB irradiated and we observed that the translocation of beta-catenin into the nucleus in the upper layer of the skin. The UV-dependent activation of beta-catenin signalling was characterized by an increase in the melanin content of melanocytes, highlighting the key role of beta-catenin in mediating the defensive pigmentation of melanocytes in response to UV stress.

IL232

Interactions between melanin and UV radiation in the genesis of cutaneous melanoma

Frances Patricia Noonan

The George Washington University, Washington, DC, United States

Cutaneous malignant melanoma (CMM) is associated with ultraviolet radiation (UV) exposure but the mechanisms are unclear. Hepatocyte growth factor transgenic (HGF/SF) mice have extrafollicular melanocytes, are hyperpigmented on the C57BL/6 genetic background, and develop melanomas recapitulating human disease after neonatal UV exposure. We delivered precise spectrally defined UVA (320-400nm) or UVB (280-320nm) at biologically relevant doses to this model and identified two UV wavelength-dependent pathways for induction of CMM and a significant role for melanin within the melanocyte in melanoma genesis. Melanoma induction by UVA required melanin and was associated with melanin-dependent oxidative DNA damage in melanocytes. In HGF/SF transgenics, melanin was largely confined to melanocytes and protective epidermal melanin was sparse, enabling direct exposure of melanocytes to UV.UVB radiation, in contrast, initiated melanoma which was associated with direct UVB DNA damage and independent of melanin. C57BL/6 mice produce black eumelanin but C57BL/6-e/e mice, with an inactivating mutation in the melanocortin-1 receptor (Mc1r), produce more phaeomelanin than eumelanin and exhibit yellow pigmentation. Phaeomelanin has been reported as more oxidative than eumelanin and has been implicated in spontaneous melanoma in a B-RAF mutant model. C57BL/6e/e-HGF mice had yellow pigmentation but produced no melanomas, spontaneously or in response to UV, although the HGF transgene and its receptor, c-Met were expressed. Melanin hyperpigmentation was not observed and there were fewer extra-follicular melanocytes than in black C57BL/6-HGF animals. Further, in heterozygous C57BL/6e/+-HGF mice, although the number of extra-follicular melanocytes and level of black hyperpigmentation were equivalent to C57BL/6-HGF, there were significantly fewer UV melanomas and only one spontaneous melanoma, indicating a pigment-independent effect. Thus an interaction between the Mc1r and HGF signaling pathways, independent of melanin production, is required for HGF-dependent melanoma. Since HGF is critical to drug resistance and treatment failure of B-RAF inhibitors this interaction may be significant in human disease.

OC233

UV wavelength-dependence studies: use of a high intensity xenon arc coupled with interference filters to produce a large field of irradiance with narrow and broadband output

Edward Charles De Fabo

The George Washington University, Washington, DC, United States

One of the challenges of wavelength dependence studies, especially in the ultraviolet (UV) spectrum, is to produce a relatively large field of irradiation with high wavelength resolution. To do this interference filters (5.08 cm x 5.08 cm), blocked from X-ray to the far IR, are coupled to a xenon arc (2.5 kW) yielding an exposure area of ~100 cm^2 . Wavelength resolutions in the UV are possible using narrow bandpass (HBW ± 1.3 nm; 260 nm-400 nm); wideband UVB, (280 nm-320 nm) or UVA-2, (320 nm-400 nm) and UVA-1, (320 nm-400 nm) filters. It is necessary to mount the filters to the xenon arc system using metal filter holders and to cool the filters to 15-20 °C during irradiation using an external A/C system and a cold water filter. A thermistor temperature probe monitors heat increase on the filters shutting down the system if excessive heat rise is detected; a shroud covering the filter-holder is used to prevent light leakage. Spectroradiometric monitoring provides accurate irradiance measurements and assessment of filter integrity. We describe the use of such a system in *in vivo* experimentation. The advantage is that it enabled production of detailed, non-overlapping wavelength analyses of *in vivo* biological action spectra in plants (pigment induction in *Neurospora crassa* from 250 nm-800 nm; De Fabo E.C. et al., *J. Plant Physiol*., 1974) and in the systemic immunosuppression of contact hypersensitivity in mice (250 nm-400 nm) implicating urocanic acid as an immunomodulator (De Fabo E.C. and Noonan F.P. J. Exp. Med., 1983); a clear differential in gene activation between UVB and UVA leading to the identification of IFN-γ as a modulator of immunosurveillance, (Zaidi, M.R. et al., Nature, 2011) and in the uncovering of two UV pathways to cutaneous melanoma identifying a critical role for UVA and melanin in melanoma (Noonan F.P. et al., Nature Commun., 2012). The disadvantages of this system are that it requires dedication of a light-tight room solely for such investigations and its cost. It is hoped that recognition of the importance of detailed wavelength resolution in carrying out *in vivo* wavelength-dependence studies may spur improvements in design and use of such a system, bringing costs down. This would enable a much broader use of high resolution, *in vivo* wavelength dependence studies and the critical advantages they provide including improvements in

photoreceptor identification; derivation of biological weighting functions for risk/benefit analysis and illuminating underlying mechanistic pathways for complex, UV/light-activated biological reactions such as gene activation and cutaneous melanoma induction.

OC234

The effect of MC1R variants and sunscreen on the response of human melanocytes in vivo to ultraviolet radiation and implications for melanoma

Elke Hacker[1], Zachary Boyce[1], Michael Kimlin[1], Leesa Wockner[2], Thomas Pollak[2], Sam Vaartjes[2], Nicholas Hayward[2], David Whiteman[2]

[1]QUT, Brisbane, Qld, Australia; [2]QIMR, Brisbane, Qld, Australia

The precise molecular mechanisms through which sunlight induces melanomas remain unclear, but there is evidence from epidemiologic, genetic and molecular studies that the effects of sunlight on melanocytes are not the same for all people. It is hypothesized that the magnitude of the proliferative response of melanocytes to sun exposure may play a role in nevi and subsequent melanoma development and that this may explain, at least in part, the variation in melanoma susceptibility observed in fair-skinned populations. We conducted a clinical trial to compare the molecular and cellular responses of human melanocytes and keratinocytes in vivo to solar simulated ultraviolet radiation (SSUVR) in 57 Caucasian participants grouped according to MC1R genotype. We found that, on average, the density of epidermal melanocytes 14 days after exposure to 2 MED SSUVR was two-fold higher than baseline (unirradiated) skin. However, the change in epidermal melanocyte counts among people carrying germline MC1R variants (97% increase) was significantly less than those with wild-type MC1R (164% increase; p=0.01). We also found that sunscreen applied to the skin before exposure to 2 MED SSUVR completely blocked the effects of DNA damage, *p53* induction and cellular proliferation in both melanocytes and keratinocytes. This study confirms the role of ultraviolet radiation in initiating melanocytic proliferation, demonstrates the effectiveness of sunscreen in preventing these molecular responses, and implicates MC1R as a key mediator in this process.

OC235

Targeting melanoma with vitamin D compounds

Katie Marie Dixon[1], Rebecca S Mason[2]

[1]Discipline of Anatomy and Histology, Bosch Institute, University of Sydney, Sydney, NSW, Australia; [2]Discipline of Physiology, Bosch Institute, University of Sydney, Sydney, NSW, Australia

Cutaneous melanoma is the most dangerous type of skin cancer, accounting for 75% of all skin cancer deaths*. The relationship between melanoma and sun exposure is interesting in that sunburn is causal while occupational sun exposure is not. Moreover, it has been suggested that patient outcome may be linked to their levels of vitamin D upon melanoma diagnosis.

Vitamin D is produced in skin following exposure to UVB from sunlight and undergoes two hydroxylation reactions to form the biologically active compound, 1,25-dihydroxyvitamin D3 (1,25D), which can mediate its effects through two different signal transduction pathways. In the well established genomic pathway, 1,25D binds the vitamin D receptor (VDR), eventually leading to up- or down-regulation of transcription of target genes. The non-genomic pathway is mediated through an alternate, putative membrane receptor.

We previously showed that 1,25D and a low calemic analog reduced UV-induced cell death and DNA damage (thymine dimers) in primary human melanocytes. Moreover, we showed that the UV-induced increase in tumour suppressor *p53* protein was further enhanced when melanocytes were incubated with 1,25D immediately after UV.

Incubation of the melanoma cell line, MM96, with 1,25D (10-9M or 10-8M) significantly ($p<0.001$) reduced cell numbers. Some melanoma cell lines have been demonstrated to be unresponsive to vitamin D and this has been linked to a failure of VDR-mediated transcription. We previously demonstrated that these concentrations of 1,25D were protective against UV-induced cell death in melanocytes without affecting numbers of melanocytes in the absence of UV.

We showed that 1,25D and a non-genomic vitamin D analog, that has limited capacity to activate the genomic vitamin D pathway, reduced UV-induced cell death ($p<0.05$) in primary human melanocytes. Moreover, the inhibition of UV-induced thymine dimers in melanocytes by 1,25D was inhibited by a non-genomic antagonist but not affected by a genomic antagonist. Thus, we have clear evidence that targeting of the non-genomic pathway in melanocytes prevents potentially mutagenic DNA damage that may lead to melanoma. We further speculate that the ability of 1,25D to inhibit growth of melanoma cells may be associated with its ability to increase expression of phosphatase and tensin homolog (PTEN).

Non-genomic vitamin D analogs, which have no demonstrated toxicity in normal cells, may prove useful in preventing and inhibiting the growth of melanoma cells.

**Melanoma Institute Australia*.

IL236

Molecular evolution and functional diversity of opsins

Yoshinori Shichida

Kyoto University, Kyoto, Japan

Opsins are retinal-based photoreceptive proteins that underlie the molecular basis of visual and non-visual photoreception in animals [1]. They are classified into at least seven groups, and we are now focusing on the Opn5 group, whose molecular analyses have begun most recently [2]. We previously showed that a chicken homolog of mammalian Opn5 (Opn5m) is a Gi-coupled UV sensor having molecular properties typical of bistable pigments. The bistable pigment forms resting (dark) and active states that are stable at physiological temperature and are able to revert to its resting state by absorbing a second photon. The bistable pigment has another characteristic, that is, the ability to directly bind to all-trans-retinal to form active state.

In the present study, we examined whether or not mammalian Opn5m has molecular properties different from those of non-mammalian Opn5m. We first confirmed that Opn5m proteins in zebrafish, *Xenopus tropicalis*, mouse and human are also UV-sensitive pigments. Then we found that, although mammalian Opn5m works as a Gi-coupled receptor pigment like non-mammalian Opn5m, a single amino acid mutation during the evolution of mammalian Opn5m led to loss of the ability to form an active state by direct binding of all-trans-retinal.

Direct binding of all-trans-retinal causes formation of the active state and elevates the G protein activation ability without light irradiation, which lowers the signal-to-noise ratio in light-dependent signaling. Therefore, by the acquisition of exclusive 11-cis-retinal binding ability of Opn5m by a single amino acid mutation, Opn5m can function as a high-sensitivity photosensor. We will show the localization of Opn5m in mammals and discuss the function of Opn5m.

[1] Shichida & Matsuyama (2009) Evolution of opsin and phototransduction. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364, 2881-2895.

[2] Yamashita et al. (2010) Opn5 is a UV-sensitive bistable pigment that couples with Gi subtype of G protein. Proc. Natl. Acad. Sci. USA 107, 22084-22089.

IL237

A novel approach for the study of protein trafficking in photoreceptor cells.

Yoshikazu Imanishi

Case Western Reserve University, Cleveland, OH, United States

Rhodopsin and peripherin/rds are essential for our vision. Rhodopsin and peripherin/rds mutations are frequently associated with inherited retina degenerative disorders. We studied the roles of sorting signals in the trafficking and mis-trafficking of these proteins. Rhodopsin and peripherin/rds were fused to a photoactivatable fluorescent protein Dendra2 and expressed in *Xenopus laevis* rod photoreceptors. Photoactivation of Dendra2 caused a shift of emission peak from green to red, allowing us to discriminate between old and newly synthesized fusion proteins. By using this photoactivation technique, we studied the dynamic events associated with the renewal of rod outer segments.

By dissecting the C-terminal tail region of rhodopsin, we discovered a new “mis-trafficking signal," which confers toxicity to rhodopsin and causes mislocalization. This mislocalization signal is activated in the absence of the VXPX cilia targeting motif. We found that the VXPX motif has two roles; (1) It neutralizes the mislocalization signal. (2) It enhances ciliary targeting at least several folds, increasing the trafficking rate of rhodopsin carrier vesicles. In the absence of the VXPX motif, mislocalized rhodopsin is eliminated from the cells through secretion of vesicles into the extracellular environment. Thus, this study revealed the roles of trafficking signals in rhodopsin trafficking, mis-trafficking, and renewal.

IL238

A2E and Lipofuscin Fluorescence in the Human RPE

Rosalie K. Crouch[1], Yiannis Koutalos[1], Anne Hanneken[2], Kevin L Schey[3], Zsolt Ablonczy[1]

[1]Medical University of South Carolina, Charleston, SC, United States; [2]Scripps Research Institute, San Diego, CA, United States; [3]Vanderbilt University, Nashville, TN, United States

The accumulation of lipofuscin in the retinal pigment epithelium (RPE) is a hallmark of aging in the eye. The best characterized component of lipofuscin is A2E, a *bis*-retinoid by-product resulting from the normal visual process. The absorption of a photon by the visual pigment generates all-*trans* retinal, which in turn is processed into A2E. *In vitro*, A2E has been shown to exhibit a broad spectrum of cytotoxic effects. We have correlated the distribution of lipofuscin and A2E across the human RPE.

Lipofuscin fluorescence was imaged in the RPE from human donors of various ages. The spatial distribution of A2E was determined using matrix-assisted laser desorption-ionization imaging mass spectrometry on both flat-mounted RPE tissue and on retinal cross sections. Our data support the clinical observations of strong RPE fluorescence, increasing with age, in the central area of the RPE. However, there was no correlation between the distribution of A2E and lipofuscin, as the levels of A2E were highest in the far periphery and decreased toward the central region.

These data demonstrate that the accumulation of A2E is not responsible for the increase in lipofuscin fluorescence observed in the central RPE with aging.

IL239

Formation of all-trans retinol in single isolated living human rod photoreceptors

Yiannis Koutalos, Chunhe Chen

Medical University of South Carolina, Charleston, SC, United States

Rhodopsin, the primary light detector of the vertebrate rod photoreceptor cells, contains 11-*cis* retinal as its chromophore. Photons absorbed by rhodopsin isomerize the 11-*cis* chromophore to all-*trans*, forming an active intermediate that initiates the reactions leading to light detection. Photoactivated rhodopsin eventually dissociates to the apo-protein opsin and all-*trans* retinal.

Opsin recombines with fresh 11-*cis* retinal to regenerate rhodopsin, while all-*trans* retinal, a highly reactive aldehyde, is removed through reduction to all-*trans* retinol. All-*trans* retinol is then transported to neighboring cells where it is used to remake 11-*cis* retinal. The reduction of all-*trans* retinal to all-*trans* retinol is catalyzed by retinol dehydrogenase and requires metabolic input in the form of NADPH. We have used the fluorescence of all-*trans* retinol to monitor its concentration in isolated human rod photoreceptors with single cell fluorescence imaging.

Single living rod photoreceptors were isolated from the retinas of human cadaver eyes (donor ages 70-90 years). Experiments were carried out at 37 °C. Isolated rod photoreceptors were bleached with long-wavelength light (>530 nm), and all-*trans* retinol formation was measured by imaging its fluorescence (excitation 360 nm; emission >420 nm). Bleaching resulted in an increase in all-*trans* retinol fluorescence, showing that the cells still contained sufficient rhodopsin to allow the measurement of all-*trans* retinol.

Formation of all-*trans* retinol proceeded with a rate constant of 0.2 – 0.4 min^{-1}, which is several times faster than in mouse rod photoreceptors. Subsequently, outer segment fluorescence declined indicating the elimination of retinol. The faster formation of all-*trans* retinol in human rod photoreceptor cells compared to mouse cells is consistent with the faster regeneration of rhodopsin after bleaching in humans. The results point to the ability of the metabolic machinery of human rod photoreceptor to supply at a high rate the NADPH necessary for the formation of all-*trans* retinol.

OC240

Cross-Protomer Interaction with the Photoactive Site in Oligomeric Proteorhodopsin Complexes

Hartmut Luecke

UC Irvine, Irvine, CA, United States

Proteorhodopsins (PR), members of the microbial rhodopsin superfamily of seven-transmembrane-helix proteins that use retinal chromophores, comprise the largest subfamily of rhodopsins, yet very little structural information is available. PRs are ubiquitous throughout all kingdoms of life and their genes have been sequenced in numerous species of marine bacteria. They have been shown to exhibit proton-pumping activity like their archaeal homolog, bacteriorhodopsin (BR). We report here the first crystal structure of a proteorhodopsin, a blue light-absorbing proteorhodopsin (BPR) isolated from the Mediterranean Sea taken at 12 m depth (*Med12*BPR). Six molecules of *Med12*BPR form a doughnut-shaped hexameric ring, unlike BR, which forms a trimer, resulting from differences in helices that are present at the interface between protomers. This also differs from a pentameric arrangement observed in two mutants of *HOT75*BPR, whose crystal structures are also reported here. The retinal tail is shifted towards helix C when compared to other microbial rhodopsins, and the putative proton-release region in BPR differs significantly from those of BR and xanthorhodopsin (XR). The most striking feature of proteorhodopsin is the position of conserved active-site His75, also found in XR, which forms a hydrogen bond with the proton acceptor, Asp97, but also with Trp34 of a neighboring promoter. Trp34 may function by stabilizing His75 into a conformation that favors an unprotonated Asp97 in the dark state, and suggests cooperative behavior between protomers when the protein is in an oligomeric form. Mutation-induced alterations in proton transfers in the BPR photocycle in *E. coli* cells provide evidence for a similar cross-protomer interaction of BPR in living cells and a functional role of the Trp34-His75 interaction in proton transport. Finally, Wat402, a key molecule responsible for proton

translocation between the Schiff base and the proton acceptor in BR, appears absent in BPR, suggesting that the ion transfer mechanism may differ between BPR and BR.

IL241

Complex climate change interactions and UV radiation: risks and opportunities for food security

Janet F. Bornman

Curtin University, International Institute of Agri-Food Security, Perth, WA, Australia

The coupled effects of climate change and UV radiation are complex and have far-reaching consequences , including for food security. The 41% increase in carbon dioxide from the use of fossil fuels since the late 1700's has increased the greenhouse effect and thus contributed to global warming. Recent measurements now confirm that carbon dioxide levels are above 400 ppm. Research also shows that stratospheric ozone and climate change are intricately linked. However, even though the Montreal Protocol in its protection of the ozone layer, and consequently avoidance of high UV radiation levels to Earth, is the most successful international protocol, impacts of an increased exposure to UV radiation can still occur independently of the ozone layer. This is because of increasing aridity in many regions, reduced cloud cover in some areas, changes in atmospheric pollution, deforestation and changes in agricultural practices, which among other factors can affect ecosystem processes and crop quality through both climate extremes and UV exposure. This presentation will provide a synopsis of some of the challenges as well as opportunities that can be explored from current and future climate projections and UV radiation.

In addition to the above, there is concern regarding the projected rise in population, reduction in natural resources and land availability. Climate variability and increased frequencies of extreme events are already having large effects on food security and the environment, and together with potential effects from UV radiation, food security thus needs to be addressed beyond the aims of only increasing production. Consequently, value-added and quality food is increasingly being proposed as the way forward for improving healthy diets and reducing environmental impacts.

IL242

From ozone depletion to agriculture – understanding the role of UV radiation in sustainable food production

Jason J Wargent[1], Brian R Jordan[2], Nigel D Paul[3]

[1]Massey University, Palmerston North, New Zealand; [2]Lincoln University, Lincoln, New Zealand; [3]Lancaster University, Lancaster, United Kingdom

Ultraviolet (UV) radiation is an energetic driver of a diverse range of plant responses, and despite historical concerns regarding the damaging consequences of UV-B radiation for global plant productivity as related to stratospheric ozone depletion, recent developments suggest that key plant responses to UV radiation may be exploitable in the context of a sustainable contribution towards the strengthening of global food production. We have characterised the responses of crop plants (e.g. *Lactuca sativa*, *Vitis vinifera*) to differing UV regimes, using a combination of modified field-scale experiments, and controlled environment approaches. It is clear that understanding and evaluating the range of agronomic outcomes mediated by UV radiation is complex, yet knowledgeable exploitation of UV can offer profound benefits. Opportunities now exist to regulate the UV environment during both pre-harvest and post-harvest phases of food production, providing life-cycle control of plant secondary metabolism for consumer health, crop plant stress resilience, resistance to pest and disease attack, and post-harvest food quality, all of which are valuable facets of consumer food acceptability, and ultimately, food security. Here we will present an overview of the prospect of this paradigm shift in photobiology, and consider those linkages between fundamental plant biology and crop-level outcomes as can be applied to plant UV response, in addition to those consequences for related biota and other aspects of food production processes.

IL243

Assessment of nutritional bioavailability of carotenoids and other phytochemicals: a key issue in analysing the potential future impact of climate change and UV radiation on food and nutrition security

Nora O'Brien

University College Cork, Cork, Ireland

There is increasing research interest in the potential effects on plants of changes in stratospheric ozone, with consequent possible increase in UV-B exposure in certain latitudes, and on the effects of increasing concentrations of atmospheric carbon dioxide, nitrous oxide and methane. Changes in plant chemical (phytochemical) composition have been reported due to variation in UV-B exposure. These changing levels of phytochemicals may have nutritional consequences – a large literature exists investigating the relationship between intake of selected phytochemicals and human health. However, any consideration of the potential health effects of phytochemicals needs to take account of their bioavailability. Bioavailability is the degree to which any chemical is absorbed into a living system where it then may have physiological effects. Traditionally, bioavailability of nutrients and phytochemicals has been assessed in human studies which are difficult and expensive to conduct giving rise to efforts to develop more rapid and inexpensive methods to assess bioavailability.

An in-vitro model for the measurement of bioavailability of nutrients from foodstuffs has been established in our laboratory. The model consists of an in-vitro digestion procedure which mimics gastric and duodenal digestion by the addition of enzymes and pH modification of the foodstuff. Carotenoid bioaccessibility is defined as the amount of ingested carotenoid that is available for absorption in the gut. Traditional food processing and preservation methods, especially thermal treatments, induce the formation of cis isomeric forms. Bioaccessibility of cis/trans isomers of carotenoids can be established using this model. The digestate is then applied to a transwell plate containing differentiated Caco-2 cells. Differentiated Caco-2 cells resemble cells of the small intestine. They are grown on a support which separates the culture dish into a top and a bottom chamber. The digested foodstuff is added to the top chamber. Nutrients present in the bottom chamber must pass through the Caco-2 monolayer and this is indicative of the amount which would be absorbed in-vivo. Effects observed in the model compared very favourably with observations from published human studies thus validating the in vitro bioavailability model.

IL244

Effects of UV-B radiation on plants; from generic stressor to specific regulator

Marcel AK Jansen[1], Karel Klem[2], Otmar Urban[2], T. Matthew Robson[3]

[1]University of Cork, School of Biological, Earth and Environmental Science, Cork, Ireland; [2]Global Change Research Center AS CR, Brno, Czech Republic; [3]University of Helsinki, Department of Biosciences, Plant Biology, Helsinki, Finland

Plant morphological responses induced by UV-B radiation (280-315 nm) are commonly reported, but major questions remain concerning the precise phenotype, underlying regulatory mechanisms, possible functions as well as consequences for plant fitness. UV-induced morphological changes include thicker leaves; shorter petioles; leaf curling, and alterations in leaf shape and width; decreases in stem elongation; increased axillary branching or tillering; altered root-shoot ratio and inflorescence

structure. It is likely that UV-induced morphological changes are underpinned by more than one molecular mechanism; at low UV-B doses through an UVR8 mediated response, perhaps fine-tuned through interactions with other environmental factors, and at high UV-B doses through a more generic stress-induced morphogenic response (SIMR) possibly involving changes in cell cycle activity. At the cellular level UV-B morphogenesis is associated with changes in cell division, elongation and/or differentiation. Various ideas have focussed on the protective function of UV-induced morphological changes with an emphasis on shading and decreased leaf penetration of UV-B. However, these ideas remain largely unproven, while not giving weight to potential trade-offs in terms of photosynthetic light capture and plant competitive abilities. For example, it has been shown that the impact of small differences in UV-induced morphology on plant-plant competitive interactions can be substantial, and this is associated with the effect of UV radiation on shoot morphology, light interception, and alternation of canopy photosynthesis for competing species. At the moment neither the relative importance of such competitive changes, nor their ecological consequences have been established. It is concluded that UV-B effects on plant morphology need to be re-appraised in the context of both their function and ecological consequences. However, to facilitate this, future research will need to disentangle the confusing and seemingly contradictory interactions that occur at the threshold UV dose where regulation and stress-induced morphogenesis overlap and where plant responses may, or may not, have a functional role and/or fitness cost.

OC245

Effect of Solar Visible and UV Radiation on Photosynthesis and Pigmentation in the cyanobacterium *Lyngbya majuscula*

Joseph Nana Annan

Dept. of Biology Education, University of Education, Winneba, Ghana

The effects of some components of solar radiation on the photosynthesis, pigmentation and phycobiliprotein composition were investigated in the marine filamentous cyanobacterium, *Lyngbya majuscula* harvested from the intertidal zone of the Biriwa coast in Ghana. The organism was exposed to unfiltered solar radiation (UV-B, UV-A and PAR), and solar radiation filtered through optical filters, WG320 (UV-A and PAR), GG400 (PAR only), and UG5 (only UV-B and UV-A). Photosynthetic oxygen production was impaired. The photoinhibition due to unfiltered solar radiation and combined UV-A and PAR were most severe. Sucrose gradient ultracentrifugation and absorption spectra of the crude extracts of *Lyngbya* indicated the presence of chlorophyll a, carotenoids, phycoerythrin, phycocyanin and allophycocyanin as the photosynthetic pigments, which were bleached under the various treatments. Generally, the phycobilins were affected most. Fluorescence measurements showed peaks that decreased significantly in amplitude and also underwent a shift towards shorter wavelengths, with prolonged exposure time, indicating that energy transfer from the accessory pigments was adversely affected. SDS-PAGE analyses of the protein profile, revealed a loss of high molecular mass proteins and that of low molecular mass, indicating a dissembling of the phycobilisomal complex and impaired energy transfer from accessory pigments to the reaction centres. The implication is that increased solar radiation may have severe consequences on the marine ecosystem.

OC246

A modelling approach to determine how much UVB radiation is available across the UK for the cutaneous production of vitamin D

Andreas Kazantzidis[1,2], Andrew Smedley[2], Richard Kift[2], Mark D Farrar[3], Jacqueline L Berry[4], Lesley E Rhodes[3], Ann R Webb[2]

[1]*Laboratory of Atmospheric Physics, Physics Department, University of Patras, Patras, Greece;* [2]*School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Manchester, United Kingdom;* [3]*Photobiology Unit, Dermatology Research Centre, Institute of Inflammation and Repair, University of Manchester, Salford Royal Hospital, Manchester, United Kingdom;* [4]*Specialist Assay Laboratory (Vitamin D), Centre for Endocrinology and Diabetes, Institute of Human Development, University of Manchester, Manchester Academic Health Science Centre, Manchester Royal Infirmary, Manchester, United Kingdom*

The UK's current nutritional guidelines assume that from school to retirement, vitamin D requirements are met by skin exposure to UVB in sunlight. However, it is well documented that at latitudes such as the UK, the sunlight in the winter can be insufficient to synthesize appreciable vitamin D levels. We are funded by the UK Department of Health to provide data to inform new national guidance on vitamin D acquisition.

In this study we use a well-established radiative transfer model to map the available UVB across the UK during the last 10 years (2003-2012). A suite of data (aerosol optical properties, surface reflectivity, cloud optical depth and coverage, total ozone column and digital elevation) derived from satellite estimates are used as model inputs to calculate the spectral UV irradiance and the vitamin D dose for different time periods.

The model-derived vitamin D doses are validated against spectral irradiance measurements at Reading (51.44N, 0.94E) and Manchester (53.47N, 2.23E). Results from more than 2500 days at each site reveal that the modeled dose is overestimated by 5% while the overall agreement is satisfactory (R^2>0.9) since the ground-based measurements are not always representative of a typical satellite measurement pixel. On a monthly basis, the overall agreement is significantly improved (bias <2%, R^2=0.99). The model results will be combined with controlled exposure studies that determined how much vitamin D is synthesized per dose of UVB in adults with different skin types. This enables an estimate of the vitamin D effective exposure available across the UK for different skin types under realistic climatological conditions. Further studies provide for a range of actual exposure patterns within the theoretical availability.

OC247

Calculations of street level solar irradiances using the Google Street View photographic database: Implementation and potential applications on photobiology studies.

Roberto Carrasco-Hernandez, Andrew R.D. Smedley, Ann R Webb

University of Manchester, School of Earth, Atmospheric and Environmental Sciences, Manchester, United Kingdom

Recent trends in atmospheric studies point towards a better understanding of the processes occurring at smaller scales, in order to create more accurate models of the weather and luminance conditions that affect human everyday life. Particularly, the urban atmosphere at street level is of special interest, both because of its relevance to a large proportion of the human population and the distinctive phenomena that can be observed in it. Many of these distinctive features in the urban micro-scale can be related to the simple concept of Sky View Factor: At any location within a city, the geometry of buildings exerts unique obstructions of the sky view, which in turn may affect not only the energy inputs and outputs at that specific location, but also aspects like human thermal comfort and human

exposure to solar radiation. Radiation exposure is usually referred to as that on an un-shaded horizontal plane e.g. for UV index forecasts. This is very different to what may be achieved in an urban canyon.
Current methods for describing the urban geometry for atmospheric purposes are not cost-effective. For example, digital 3D mapping of cities will return wide geographic coverage but at the cost of low resolution and undetailed representation of urban canyons. On the other hand "fisheye" photographs of single locations will allow detailed descriptions at the cost of narrow geographic coverage. Nevertheless, the Google Street View TMweb app possesses a large, free-access database of street photographs. Such database is readily available to be used as reference for describing urban geometries. Results would comprise accurate descriptions of single locations plus widespread coverage of populated geographic regions. The use of digital image processing software (Hugin, ArcGIS, Maxent) allows the semi-automatic reconstruction of urban geometries from Google Street View photographs. The aforementioned tools in combination with solar radiation calculation algorithms (Rayman, Libradtran) assist in the modelling of solar irradiances (either total, spectral, or spectrum weighted irradiances) that can be calculated on the basis of the visibility of the solar disc (direct irradiances) and the sky view factor affecting the diffuse component of irradiance. We will illustrate the output of such models for selected locations and show how the street level irradiances can be very different to the full sky view measurements or models on which, for example, the UV index is based. Potential applications of these models include street photovoltaic generation, daylight & heat loading in the energy budget calculations of cities, and human exposure to biologically active radiation.
Keywords: Urban Canyons, Sky View Factor, Solar Irradiance, Google Street View.

OC248

Suggestions for standardisation of biologically weighted units for human skin UV exposure

Mario Blumthaler[1], Richard McKenzie[2], Ann Ruth Webb[3]
[1]*Innsbruck Medical University, Division for Biomedical Physics, Innsbruck, Austria;* [2]*National Institute of Water and Atmospheric Research, Lauder, New Zealand;* [3] *University of Manchester, School of Earth, Atmospheric and Environmental Sciences, Manchester, United Kingdom*

Physical units of radiation are defined for irradiance and radiant exposure, but it is also necessary to define units, which take into account specific biological reactions. Two of the most significant biological reactions of human skin due to UV exposure are discussed here in detail: erythema and production of Vitamin D. The response of the human skin to spectral UV radiation is described by the action spectra for erythema and Vitamin D, which are already defined by CIE. These action spectra are dimensionless and usually normalized to the maximum of the reaction. The biologically relevant irradiance is determined by multiplication of the spectral irradiance with the respective action spectrum and then integration over all effective wavelengths. The result still has the unit of irradiance and is called 'erythemally weighted irradiance' (UV_{Ery}) or 'Vitamin-D weighted irradiance' (UV_{VitD}). For UV_{Ery} a new unit is already in use for public health information, the 'UV-Index', which corresponds to $40*UV_{Ery}$, when UV_{Ery} is expressed in Wm^{-2}. For the final biological reaction the product of irradiance with time (i.e. radiant exposure or dose) is the significant quantity. To quantify this dose for erythema, the 'standard erythema dose' (SED) is defined as 100 Jm^{-2} of erythemally weighted dose. A further quantity which takes account of the different sensitivity of skin types is the 'minimum erythema dose' (MED), which ranges between 2 and 20 SED dependent on skin type.
By analogy to the derived units for erythema we suggest the definition of the corresponding units for Vitamin D production:
'*standard Vitamin D dose*' (SDD) = 100 Jm^{-2} of Vitamin D weighted dose.
'*minimum Vitamin D dose*' (MDD): should be equivalent to the daily oral intake of 1000 IU. The conversion factor is based on empirical studies and amounts to about 0.2 SDD for full body exposure and skin type II.
For the production of Vitamin D one has to consider in addition that the required dose for the end biological effect is proportional to the exposed skin area (this is not the case for erythema). The definition above refers to full body exposure, but the dose (the MDD) will increase if the skin area exposed decreases. Furthermore the relation between MED and MDD depends on the spectrum of the irradiance source, and thus for solar radiation the relationship depends on location, time of year and time of day.

OC249

The Wallow&Swallow-model for vitamin-D status dynamics

Arjan van Dijk, Harry Slaper, Henk van Kranen
RIVM, Bilthoven, Netherlands

We have developed a conceptual model linking vitamin D status to input from both oral intake and skin production after exposure to UV. The Wallow&Swallow-model has been mathematically implemented in FORTRAN and it can be used to evaluate scenarios. Some parameters in the model have been successfully estimated on the basis of a variety of experimental data reported in literature. An example: the turn-over rate of vitamin D in the body has been modeled as a function of the vitamin D status, using the findings from 41 different studies on the response to changes in oral intake. Other parameters remain to be estimated. Two of the more interesting parameters in this respect are: 1) the efficiency of the photochemical conversion in skin and 2) the role of adipose tissue: for example: does buffering from fat dominate vitamin D dynamics in winter? The mathematical formulation of the Wallow&Swallow-model, which is operated with a typical time-step of 1 minute, is purely incremental. This means that it is sufficient for the model to know the current status of the body and the current inputs to allow for updating the status some timestep later. This conceptual set-up was adopted to facilitate the inclusion of non-linear dynamics ("synergy" between different effects) in the model. An advantage in practice is that it also facilitates the analysis of scenarios with behavioural feed-back loops, e.g. when people decide to evade the sun on the basis of their acquired erythemal status. Alternatively, it can be used to see the impact of external factors affecting the system, e.g. dietary regime, liposuction or blood transfusion. The Wallow&Swallow-model includes a radiative transfer module to estimate time-resolved location-specific irradiances associated with exposure scenarios on the basis of a data-base of historical remote-sensing maps (NIMBUS, EP-TOMS, SCIAMACHY, OMI) for ozone and cloudiness. In this presentation we will introduce the Wallow&Swallow-model, show some examples of applications and discuss uncertainties and future developments.

PL301

Towards the rational design of fluorescent proteins tailored for super resolution imaging

Johan Hofkens, Peter Dedecker, Sam Duwé, Benjamien Moeyaert
KU Leuven, Leuven, Belgium

In recent years, the development of new imaging techniques has allowed researchers to push the boundaries of fluorescence microscopy past the diffraction limit. Many of these techniques require the use of 'smart labels' such as photo-activatable and reversibly photoswitchable fluorophores. Although their performance is adequate for most basic visualization experiments, further improvement is needed if we want to push the state of the art past this basic level of 'straightforward super-resolution imaging'.

We previously reported on NijiFP, a four-way highlighter FP that is green-to-red photoconvertible and reversibly photoswitching in both states. In the present work, we engineered a similar optical highlighter probe by engineering photoconversion properties into the well-known reversibly photochromic protein Dronpa. We made ffDronpa, a Dronpa mutant that is formed up to three times as fast as Dronpa, while retaining the photochromic features of Dronpa. Using rational and random mutagenesis, we transformed ffDronpa to pcDronpa. This mutant combines Dronpa's photochromism with the feature of being photoconvertible to a red state. pcDronpa was studied in detail and was applied in several microscopic settings. We have shown that atomic level structure determination plays a pivotal role in linking spectroscopic characteristics to structural features
Next, we developed new reversibly switchable fluorescent proteins based on rsEGFP with improved performance in structurally strained fusion constructs and general improved behavior at 37°C. Through the use of a disordered bait protein, which hampers the correct formation of a functional fluorescent protein, in combination with directed and random mutagenesis, we pushed our rsFPs further towards structural perfection. Several mutants show increased maturation/folding when expressed at 37°C or when put under strain by the bait protein, while retaining the specific photophysical properties and switching characteristics of rsEGFP. Other interesting variants show shifted fluorescence spectra and altered photochromic behavior. These results work demonstrates that it is possible to improve existing rsFPs on the biological/structural level while retaining their full capacity on the photophysical level. The development of these improved rsFPs will allow for more demanding imaging experiments to be carried out and will create more opportunities for the development of 'smart biosensors' because of the increased performance in fusion constructs.

IL302
Photoimmunosuppression and the role of UVA

Gary Halliday, Scott Byrne, Guy Lyons, Diona Damian
University of Sydney, Sydney, Australia

UV radiation suppresses immunity in both humans and mice. UV suppresses the induction of primary immunity, whether the antigen is at the site of UV or a site distal to UV. It can also suppress the reactivation of memory responses and immunity in other organs. Different mechanisms appear to be responsible for UV suppression of immunity in the skin and peripheral lymphoid organs. Hence photoprotection of skin and internal immunity need to be considered. UVB and UVA are most likely co-carcinogens for human skin cancer and both wavebands can induce skin cancer in animal models. Reactive oxygen species (ROS) oxidize guanine to 8-oxo-7,8-dihydroguanine (8-oxo-dG), which is thought to be the predominant DNA damage formed in response to UVA. This is repaired by 8-oxoguanine-DNA glycosylase 1 (OGG1). Anti-OGG1 staining of human skin showed the highest expression in the superficial epidermal layer. UVA-induced 8-oxo-dG was repaired more rapidly in the upper layer of human skin compared to the lower layers. This indicates that weaker expression of the OGG1 enzyme in the basal cells of human epidermis may result in a lack of DNA repair in these cells and therefore accumulation of UVA-induced oxidative DNA mutations. Basal cell carcinoma, like basal keratinocytes, expresses low levels of OGG1 and accumulates large amounts of 8-oxo-dG. UV suppression of immunity is a critical biological mechanism responsible for skin cancer. Both UVB and UVA are potently immunosuppressive in humans, enabling mutated cells to grow unchecked. Our recent action spectrum shows two immunosuppressive peaks, one in the UVB range at 300 nm and a second in the high wavelength UVA at 370 nm. The action spectra for UVA-induced immunosuppression and ROS production are similar, suggesting that ROS production may be responsible for UVA immunosuppression in humans. This is most likely responsible for the requirement for a sunscreen to have good UVA absorption in order to protect the immune system. A common thread in damaging effects of UV on the immune system and DNA is suppression of glycolysis and ATP production. This can be overcome with nicotinamide (vitamin B3). Nicotinamide can protect humans from both UVB and UVA-induced immunosuppression and therefore appears promising for photoprotection. There is now accumulating evidence that the role of UVA in causing skin cancer in humans has been underestimated, and ROS and cellular energy production may be critically involved.

IL303
Redox-directed interventions targeting solar photodamage: Nrf2 and beyond.

Georg Thomas Wondrak
University of Arizona, College of Pharmacy and Arizona Cancer Center, Tucson, AZ, United States

Exposure to chronic solar UVA-radiation is a causative factor in cutaneous photocarcinogenesis and photoaging. Cumulative evidence suggests that UVA-induced photodamage occurs by photooxidative mechanisms mediated by reactive oxygen species (ROS). Advanced redox-directed strategies that antagonize specific mechanisms underlying photooxidative stress may therefore offer significant photoprotective benefit. Here we describe our recent research efforts aiming at the identification of (i) novel endogenous photosensitizers, (ii) novel cutaneous key targets modulated by photooxidative stress, and (iii) improved strategies for redox-directed photoprotective intervention in human skin. As a result of screening a focused compound library of skin chromophores for UVA-driven photodynamic activity targeting HaCaT keratinocytes and Hs27 dermal fibroblasts, we have identified ⟨-dicarbonyl- (including the glycolytic metabolites methylglyoxal and pyruvate) and indolocarbazole-chromophores as novel endogenous skin photosensitizers active in the low nanomolar concentration range. In an attempt to identify upstream molecular targets modulated by UVA-induced photooxidative stress we employed DIGE-proteomics demonstrating that inactivation of cysteine proteases cathepsin L (CTSL) and B (CTSB) underlies UVA-induced autophagic-lysosomal blockade in dermal fibroblasts characterized by accumulation of Lamp1, p62, LC3-II, and lipofuscin. The causative role of UVA-induced cathepsin inactivation in fibroblast-directed functional impairment was further confirmed by pharmacological (CA074Me) and genetic (CTSB/L-directed siRNA) target modulation. Consistent with the established role of Nrf2 as a master regulator of cellular antioxidant gene expression, we observed the exquisite sensitivity of Nrf2 KO versus Nrf2 wildtype mouse embryonal fibroblasts to UVA-driven photosensitization and lysosomal-autophagic impairment with cathepsin inactivation and lipofuscin accumulation. A luciferase-based reporter gene assay was used as a primary screen for the identification of novel agents that modulate the Nrf2-Keap1 signaling pathway for protection against photooxidative stress in dermal fibroblasts. Pharmacological Nrf2 activation using a novel medicinal plant-derived phenanthrenequinone biofactor blocked UVA-induced dermal oxidative stress, lysosomal impairment, and photodamage in cultured dermal fibroblasts and a full thickness human skin reconstruct.

IL304
New directions in formulation of sunscreens

Dominique Moyal
L'OREAL RESEARCH & INNOVATION, Asnières-sur-Seine, France

Since the last 40 years, leisure activities and style of living have noticeably changed and outdoor activities have significantly increased. Therefore having a natural tan has become a symbol of a healthy and active life for many consumers. One of the most

important pastimes during summertime is sun bathing which often leads to overexposure.
Now it is well known that overexposure to sunlight has negative consequences on human skin: the remaining UV radiation (UVR) reaching the earth can induce dramatic changes in the physiological state of the skin.
Consumers are more aware of the danger of sun light exposure and now, use of sun protection products is essential during sun exposure. The product efficacy must be adapted to conditions of use and to sunshine period.
When designing a sunscreen product formulation, essential characteristics must be fulfilled:
(1) It must be efficient against acute and chronic damages of sun;
(2) It must be heat-stable and also photo-stable so as to ensure durability of the protection;
(3) It must be persistent to sweat, bathing and rubbing off,
(4) It must be attractive, pleasant and easy to use to promote frequent applications.
A sun care product is based on the combination of active ingredients i.e. UV filters and a vehicle to ensure a uniform spreading on the skin.
The efficacy of the formulations depends on a wide variety of factors.
First, the formulator must consider the chemical structure and the concentration of the filtering agents to design a specific profile of absorption and to reach a high level of protection. As far as broad and well-balanced UVB/UVA protection is concerned, there is a specific issue in formulating UVA photostable filtering systems.
Synergistic combinations of UV filters are designed to obtain high protection factor (SPF and UVA-PF) and to reduce the total concentration of UV filters.
Different strategies can be used to improve the solubility and the photostability of the UV filters.
Moreover, there can be significant efficacy differences between formulations containing the same filtering system. This is primarily driven by several parameters including thickness, opacity and uniformity of the film applied on the skin.
To develop high quality sun care products we must understand the physico-chemical principles - not only the UV absorbance/chemistry of the actives - but also the effects and interactions and the behaviour of the vehicle component in order to promote an uniform film spreading which is part of the overall efficacy of the finished product.
Different strategies based on non absorbing material can be used to correct the spreading and boost the efficiency of the product.

IL305
Sunscreens and the US FDA

Henry W. Lim
Dermatology- Henry Ford Hospital, Detroit, MI, United States

On June 17, 2011, the US Food and Drug Administration (FDA) released the Sunscreen Final Rule, which was implemented on Dec 17, 2012. Highlights of this Final Rules are:

- For the first time, there is an explicit guideline in the US on the assessment of UVA protection by sunscreens, specifically, an in vitro critical wavelength test. It is a pass/fail system at 370 nm. Products that have a critical wavelength of ≥ 370 nm, and have SPF>15, can then claim "broad spectrum" label.
- The "broad spectrum" label has to be of the same font size and on the same line as the SPF label.
- Broad spectrum products with SPF≥15 are allowed to state on label: "if used as directed with other sun protection measures, (sunscreens) decrease the risk of skin cancer and early skin aging caused by the sun."
- Products that are broad spectrum, but with SPF<15, or those with SPF ≥15 but not broad spectrum: they can only state on the label: "only to prevent sunburn."
- Water-resistant labeling will be stated as "water resistant (40 min)" or "water resistant (80 min)". "Water/sweat proof" labeling will NOT be allowed.
- In addition, the FDA is seeking comment under a Proposed Rule on SPF 50 or above. FDA is also seeking additional safety data on spray form of sunscreen products. Furthermore, FDA proposes that wipes, towelettes, powders, body washes and shampoos are ineligible for review under the sunscreen monograph process; FDA is seeking comments on this proposal.

OC306
Transformer Material in Dynamic UV Protection

René Peter Scheurich, Thomas Rudolph
Merck KgaA, Darmstadt, Germany

State of the art suncare products are offering protection of human skin against solar radiation. However, many of the sunscreens known and used in such products are prone to photo-induced decomposition over use time. Photostability is usually correlated to a behaviour that corresponds to loss of UV absorption capacity vs. time. Herein we present materials that create a new understanding of photostability. Our new transformer materials are converted into known UV filter compounds by sunlight. These materials are no UV-absorbers prior to irradiation. Upon being exposed to sunlight similar radiation (SSR) they are being transformed into UV filters. The conversion is radiation dose dependant
Key feature of the new transforming materials is a direct response to the radiative input on a consumer thus enabling a dynamic UV protection of human skin for the first time. The concept adds safety in use to the consumer and for the first time ever allows to generate anti UV products whose UV protection capacity increases over use time.

OC307
Essential minimum textile and dermal protection for outdoor workers in mid-latitudes – in adaptation of the ICNIRP-safety concept to artificial UVR at workplaces

Peter Manfred Knuschke[1], Marco Janssen[2], Guenter Ott[2]
[1]TU Dresden, Medical Faculty, Dept. of Dermatology, Dresden, Germany; [2]BAuA Dortmund, Dortmund, Germany

For the 8h-workday at workplaces with the risk of exposure by artificial UV-sources the ICNIRP recommended spectral threshold values (taken over e. g. by the EU-Directive 2006/25/EC "Artificial Optical Radiation"). This spectral threshold values are an "artificial action spectrum" that envelops action spectra of the acute UV injuries to the eyes (photoconjunctivitis, photokeratitis) and to the skin (UV-erythema) with a safety factor 2.5 to 3 for the 8h-workday. The basic consent of the ICNIRP safety concept is that UV-exposures less than 30 % of the acute reaction threshold would not result in chronic effects – even if repeated every 24 h.
Basing firstly on this ICNIRP safety concept to repeated UV-exposures of workers and secondly on analysis of the real solar UV-exposure conditions of outdoor workplaces we propose minimum requirements on the ultraviolet protection factor (UPF) of closing worn by outdoor workers and SPF of dermal skin protection*).
In practice, organizational measures or/and technical measures to reduce solar UV-exposure at outdoor workplaces are often not possible to realize. Therefore, closing, cap and sun protection glasses have the function of individual protection measures.
Depending on (a) the real UV-exposure per workshift, (b) the body distribution of the solar radiation and (c) the body distribution of the skin sensitivity to UV-radiation: we determined for different body sites minimum required UPF for textiles covering the skin areal as well as the SPF for uncovered skin areas, to realize UV-exposure in each skin area less than 30 % of the MED. To derive the UV-exposure data per workshift we analysed half hour erythema dose datasets (2000-2008) of seven stations of the German solar UV-monitoring measurement network (sUVMonNet). The body distribution of solar UV-

exposure of outdoor workers was measured under real working conditions seasonally in previous research projects. The erythemal skin sensitivity was derived from literature and own experience in photodiagnostics.
In the mid-latitudes (45°-60° N; Germany: 47°-55° N) for the body sites a erythemal UV-exposure < 30 % MED (skin type II) requires at least UPF: 20 (chest, back), 50 (shoulder), 6 (thigh) and for uncovered skin parts (repeated) SPF: 20 (face, neck, nape) and 10 (hands, arms, legs).
*) German Federal Institute of Occupational Safety and Health (BAuA) support-N° F2036 – Research project: "Protection components to reduce solar UV-exposure of outdoor workers"

OC308

Cyclooxgenase inhibitors: agents of photoprotection or photoexacerbation?

Suzanne Margaret Pilkington[1], Shobha Kudva[1], Alex Kendall[2], Sharon Murphy[2], Anna Nicolaou[2], Lesley Elizabeth Rhodes[1]
[1]University of Manchester, Manchester, United Kingdom; [2]University of Bradford, Bradford, United Kingdom

UVR induces acute skin inflammation, characterised by erythema, dermal leukocytic infiltration and upregulation of pro-inflammatory eicosanoids. Metabolism of arachidonic acid (AA) is enhanced, with notable production of vasodilator prostaglandin E_2 (PGE_2) and chemoattractant 12-hydroxyeicosatetraenoic acid (12-HETE), via the cyclooxygenase-2 (COX-2) and 12-lipoxygenase (12-LOX) pathways, respectively. While COX inhibition reduces AA conversion to PGE_2, decreasing UVR-erythema, we address whether the enhanced availability of AA for metabolism by LOX may conversely exacerbate the inflammation.
In 12 healthy volunteers (phototype II/III; 20-39y; 8 female), suction blister fluid (n=10 subjects) and/or skin punch biopsies (n=3 subjects) were sampled from UVR-exposed (3MED; 24 and 72h post-challenge) and unexposed buttock skin with and without topical indomethacin gel applied immediately post-UVR. Blister fluid eicosanoid levels were analysed by LC-MS/MS, and skin leukocytic infiltrate (neutrophil elastase, CD3, CD8) and 12-LOX expression by immunohistochemistry. UVR-induced erythema (Hb Index) was quantified pre-sampling via spectrophotometry. Topical indomethacin reduced mean UVR-erythema at both 24h (1.1 (SEM 0.1) vs 1.7 (0.2)) and 72h (0.9 (0.6) vs 1.3 (0.9)) post-exposure compared to untreated skin (both $p<0.001$). Median (IQR) PGE_2 level was also reduced after indomethacin at both 24h (1.3 (2.2) vs 12.6 (47.3) pg/µl, 95% reduction, $p<0.05$) and 72h (0.5 (0.7) vs 3.3 (17.7) pg/µl, 86% reduction, $p<0.01$) post UVR. In contrast, levels of 12-HETE increased after indomethacin at both the 24h (94.5 (70.0) vs 37.5 (46.1); 60% increase) and 72h time-points (77.0 (79.2) vs 50.6 (53.9) pg/µl; 53% increase; both $p<0.05$) compared to untreated skin, while 12-LOX expression showed little change. Immunostaining also indicated increased dermal UVR-induced neutrophils after indomethacin compared to untreated skin at 24h (mean 27.3 (SEM 20.5) vs 11.9 (3.9)) and 72h (12.4 (6.3) vs 6.4 (1.3)) post UVR. Similarly, CD3+ and CD8+ T cells increased in treated *versus* untreated skin, particularly at 72h; CD3+: 70.9 (5.2) *vs* 38.7 (4.4), and CD8+: 36.5 (9.0) *vs* 15.3 (6.7).
Thus, while COX inhibition reduces the erythemal component of sunburn, we found evidence that the UVR-induced inflammatory cell infiltrate is exacerbated, which may be attributable to higher levels of the potent chemoattractant 12-HETE. Further studies are ongoing to confirm these findings.

OC309

Sunscreens Have Failed to Prevent Skin Cancer, What Went Wrong?

Robert M. Sayre[1,2,3], John C. Dowdy[1]
[1]Rapid Precision Testing Laboratories, Cordova, TN, United States; [2]Department of Dermatology, University of Tennessee Center for the Health Sciences, Memphis, TN, United States; [3]Department of Physics, University of Memphis, Memphis, TN, United States

In 1975 the US National Institutes of Health published "Measurements of Ultraviolet Radiation in the United States and Comparisons with Skin Cancer Data." They reported half hour measurements for a year of solar erythemic effective UV data from 10 US population areas along with skin cancer surveys for four of the centers. They concluded that the risk to non-melanoma skin cancer was dependent upon the amount of erythemic risk in different population centers. Examination of the accumulative 1 year of data suggested that if an SPF 2 product were used daily by individuals at risk to skin cancer then the incidence of non-melanoma skin cancer for women could be expected to be reduced by more than 70% and for men by more than 90%. In 1975 the average sunscreen product sold was between an SPF 2 and 4 by today's labeling. In fact each year since, sunscreens have become progressively more effective until today they generally range from SPF 30 to 100. Today only the two approved physical sunscreen agents will remain on the skin's surface while all other active agents are formulated to readily and rapidly penetrate into the skin. Recently a number of the currently approved sunscreen agents have been shown to be anti-inflammatory agents. This amounts to perhaps half of all approved sunscreen agents. Another currently approved anti-inflammatory, analgesic agent was found also to be an approved sunscreen. It is suggested that today all sunscreen testing needs re-examination as neither in-vivo nor in-vitro testing has any validity when the simple presence of an anti-inflammatory will confound and even conceal acute responses to UV injury including sunburn redness and pain.

IL310

Fifty Years of Research in Photomovements

Francesco Lenci
Istituto di Biofisica CNR, Pisa, Italy

A wide variety of 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/biochemical signal which can be recognized, elaborated and transduced by the cell. Thanks to this photosensory capability, spatial and temporal variations in the photic environment can elicit modifications of movement patterns. In other words, light constitutes an information signal which controls the movement and eventually drives the cells into the best environmental niches for their growth, survival and development. In the beginning mainly behavioural responses of cell populations were investigated using the so-called "photaxigraphs". Single-cell studies, using manual and then computer-assisted tracking of cells trajectories allowed discriminating among different "photobehaviours" of the photoresponsive microorganisms. Action spectra determination, both in cell population and single studies, were widely used with the aim of identifying the photoreceptor pigment. *In vivo* microspectroscopies and confocal microscopy are nowadays currently employed to investigate structural properties as well as light-induced reactions of photosensing units in their physiological molecular environment. Chemical surgery (use of specific drugs) has been used to identify the "dark steps" of the transduction chain, but molecular biology and genetic engineering are playing a key role in giving important contributions. From what concisely reported above, it should be evident that only a sound interdisciplinary approach can actually

aim to clarify “The Problem”: understanding the molecular basis of photosensory processes in aneural cells, like microorganism, from the absorption of the photon in the photoreceptor system to the motor response of the cell, through a series of light dependent as well as dark reactions.

IL311

Phytochrome Transgenes for Improving Turf Grass Traits

Mayank Anand Gururani[1], Markkandan Ganesan[1], Yun Jeon Han[2], Hyo Yeon Lee[1], Jeong Il Kim[2], Pill Soon Song[1]

[1]*Faculty of Biotechnology, Jeju National University, Jeju, Republic of Korea;* [2]*Kumho Life Science Laboratory, Chonnam National University, Gwangju, Republic of Korea*

Phytochromes are a group of photoreceptors that mediate the plant responses to red and far-red light and regulate photomorphogenesis in plants. Phytochrome engineering has been extended to several crops such as tobacco (Robson et al., 1996), Arabidopsis (Margaret et al., 1991; Jason et al., 1994), rice (Garg et al., 2006), sweet potato (Yi et al., 2007; Kim et al., 2009) and potato (Thiele et al., 1999) with varying degree of success and different objectives. Shade avoidance responses are regulated by the phytochromes and it has a vital adaptive significance in plants. Recently, we introduced a mutant *Avena sativa* PHYA gene encoding phytochrome A with a blocked light-signal attenuation phosphorylation site that led to a stronger shade avoidance tolerance and enhanced photosynthetic efficiency in Zoysiagrass. The mutant Zoysiagrass warm season grass, exhibited wider and greener leaves, increased number of tillers and delayed senescence (Ganesan et al., 2012). Among the light-stable phytochromes, phyB regulates the shade avoidance traits such as elongated hypocotyls, stem and leaves, early flowering and higher apical dominance under high red/far-red light. Current research in our laboratory is focused on the development of photosynthetically efficient, shade-tolerant transgenic Zoysiagrass lines by introducing WT and mutant PHYB genes from rice. The mutant rice PHYB created by site-directed mutagenesis encodes phytochrome B in which a Tyr- to His- mutation was introduced (Fischer et al., 2005; Su and Lagarias, 2007). Similar efforts of producing engineered Zoysiagrass harboring mutant PHYB genes from Arabidopsis and Brachypodium species with Tyr- to Val- mutations will form a part of our future research with an aim to produce environmentally friendly and commercially beneficial turfgrass cultivars.

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IL312

Fidelity of Adaptive Phototaxis

Raymond E. Goldstein

University of Cambridge, Cambridge, United Kingdom

Along the evolutionary path from single cells to multicellular organisms with a central nervous system are species of intermediate complexity that move in ways suggesting high-level coordination, yet have none. Instead, organisms of this type possess many autonomous cells endowed with programs that have evolved to achieve concerted responses to environmental stimuli. In this talk I will describe how experiment and theory have been used to develop a quantitative understanding [1] of how cells of such organisms coordinate to achieve phototaxis, by using the colonial alga *Volvox carteri* as a model. It is shown that the surface somatic cells act as individuals but are orchestrated by their relative position in the spherical extracellular matrix and their common photoresponse function to achieve colony-level coordination. Analysis of models that range from the minimal to the biologically faithful shows that, because the flagellar beating displays an adaptive down-regulation in response to light, the colony needs to spin around its swimming direction and that the response kinetics and natural spinning frequency of the colony appear to be mutually tuned to give the maximum photoresponse. These models further predict that the phototactic ability decreases dramatically when the colony does not spin at its natural frequency, a result confirmed by phototaxis assays in which colony rotation was slowed by increasing the fluid viscosity.

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IL313

Early signalling steps leading to hypocotyl phototropism in *Arabidopsis*

Christian Fankhauser[1], Tobias Preuten[1], Emilie Demarsy[1], Tim Hohm[2], Paolo Schumacher[1]

[1]*Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland;* [2]*Department of Medical Genetics, University of Lausanne and Swiss Institute of Bioinformatics, Lausanne, Switzerland*

Phototropin blue-light receptors (phot1 and phot2) in *Arabidopsis* activate a range of light regulated responses, including phototropism, leaf movements, stomatal opening, leaf expansion, and chloroplast movements. Those responses generally serve to optimize photosynthesis and allow the plant to adapt to changing light environments. Phototropins are light-regulated protein kinases that are broadly expressed and present at the plasma membrane in the dark. Blue light induces their protein kinase activity and leads to internalization of a fraction of the photoreceptor. In order to understand the steps leading from photoreceptor activation to asymmetric hypocotyl growth leading to phototropism we are addressing the following questions: (1) Where does phot1 perceive the light signal that activates phototropism? Does the photoreceptor act autonomously, or does the response involve transportation of a signal from the site of light perception to the site of action? (2) Is light-induced phot1 translocation from the plasma membrane to the cytosol a mechanism of desensitization or is its transport into the cytosol essential for signalling? (3) What are the substrates of phot1 kinase activity and how does phosphorylation regulate these targets. I will discuss our latest findings on these questions.

OC314

Photophysical determinants of blepharismin, the photoreceptor pigment of the ciliate *Blepharisma japonicum*

Ranieri Bizzarri, Francesco Ghetti, Giovanni Checcucci

Consiglio Nazionale delle Ricerche, Istituto di Biofisica, Pisa, Italy

The heterotrichous ciliate *Blepharisma japonicum* dwells in the debris at the bottom of water bodies where light is sparse or virtually non-existent. When *B. japonicum* casually swims in lighted areas, a series of step-up photophobic responses help this microorganism to revert back to shadowed areas by means of a trial-and-error process [1]. This photobehaviour is driven by the benzodianthronic molecule blepharismin, which is distributed all over the cell body either free or confined in pigment granules, colouring the ciliate in red [2] Blepharismin absorption spectrum in the visible range shows three characteristic bands peaked at about 580, 540, and 490 nm [3]. A pigment–protein complex has been isolated with an apoprotein of molecular weight of about 200 kDa [4]. The pigment is not covalently bound to the proteic framework but it is presumably inserted in a molecular pocket of the protein [5]. In this work, we started a systematic analysis of photophysical determinants of blepharismin pigment (both in the native "red" and in the photoconverted "blue" forms) such as emission spectra profile and fluorescence lifetime emission. These determinants were investigated by confocal microscopy in *B. japonicum* cells, in the perspective that the optical behaviour of the molecule in any spatial location is strictly connected to its biological functions. Remarkably, the use of "phasor" approach

made data analysis [6] particularly simple and helped to unveil the spatial variability of the optical response of blepharismin in the cell.

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IL315

Cryptochrome photoreceptor mechanism of activation

M. Ahmad[1], D. Robles[1], C. Englehardt[2], A. Batschauer[3], R. Bittl[2]

[1]*University of Paris, Paris, France;* [2]*Freie Universitaet Berlin, Berlin, Germany;* [3]*Phillips-Universitaet Marburg, Marburg, Germany*

Cryptochromes are blue light absorbing flavoproteins that mediate many important signaling functions in plants including photomorphogenesis, the initiation of flowering, and the entrainment of the circadian clock. The photochemical activation of plant cryptochromes (cry1 and cry2) has been proposed to involve flavin radical formation via an electron transport chain comprising 3 evolutionarily conserved tryptophan residues known as the 'trp triad'. Here we show the surprising result that 'trp triad' mutants of cry2 that are photochemically inactive *in vitro* do in fact retain significant photochemical activity *in vivo*. Intact wild type cry1 and cry2 proteins, in turn, show greatly increased photochemical efficiency in vivo as compared to isolated proteins. The seeming discrepancy between in vivo and in vitro photochemical activity is resolved by both optical and whole cell EPR spectroscopic measurements showing that small cellular metabolites serve as 'activators' of cry function. We conclude that cry photochemistry can be profoundly modified by the cellular environment, and that cry2 biological activity is consistent with a requirement for flavin photoreduction in a biological context. The possible roles of metabolite activators in signalling will be further developed. The implications of this mechanism for conformational within the protein, for activation of animal type cryptochromes, and for cryptochrome responsivity to the geomagnetic field will be discussed.

IL316

Stimulated Raman Imaging

Herman L Offerhaus, Erik T Garbacik, Andrew L Fussell
University of Twente, Enschede, Netherlands

Coherent anti-Stokes Raman Scattering (CARS) microscopy has emerged as a microscopy method that offers high spatial resolution (~1 micron), fast imaging (1s per image) for samples with reasonably high local concentrations. I will present our CARS setup with its characteristics and limitations and show results on CARS microscopy for pharmaceutics and medical applications. Our setup is based on an Optical Parametric Amplifier (OPO) which also allows for detection of the phase of the CARS process. The OPO wavelength can be scanned to obtain spectral information over a wider range which has proven to be quite useful for the discrimination of different crystal polymorphs.

IL317

Porphysome Nanotechnology: Explore New Frontiers of Cancer Imaging

Gang Zheng
University of Toronto, Toronto, ON, Canada

We recently discovered ‘porphysomes’, the first all-organic nanoparticles with intrinsic multimodal photonic properties. They are self-assembled from porphyrin-lipid building blocks with extremely high porphyrin packing density (>80,000 per particle), resulting in both ‘super’-absorption and structure-dependent ‘super’-quenching, which, in turn, converts light energy to heat with extremely high efficiency, giving them ideal photothermal and photoacoustic properties. Upon porphysome nanostructure dissociation, fluorescence of free porphyrins is restored to enable low background fluorescence imaging. In addition, metal ions (e.g., radioactive copper-64) can be directly incorporated into the porphyrin building blocks of the preformed porphysomes thus unlocking their potential for PET, MRI and radiation therapy. In a similar manner to liposomes, the large aqueous core of porphysomes could be passively or actively loaded with drugs, opening up a new avenue for image-guided drug delivery. By changing the way porphyrin-lipid assembles, the ultra small porphyrin nanoparticle (<20nm) and the large porphyrin shell microbubbles (~2m) were developed to further expand the purview of porphyrin-based cancer imaging. The simple yet “one-for-all” nature of porphysomes confers high potential for clinical translation.

IL318

In Vivo Rapid Cancer Detection Based on Rationally Designed Activatable Fluorescence Probes

Yasuteru Urano
Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Fluorescence imaging is one of the most powerful techniques currently available for continuous observation of dynamic intracellular processes in living cells. Suitable fluorescence probes are naturally of critical importance for fluorescence imaging, and we have succeeded to construct several versatile rational design strategies for novel fluorescence probes based on the concept of photoinduced electron transfer [1,2] and intramolecular spirocyclization [3,4].

Very recently, we have succeeded to develop various novel protease probes which were applicable for living cell system [4]. For example, gGlu-HMRG, a novel spirocyclized rhodamine-based fluorescence probe for gamma-glutamyltranspeptidase (GGT), which is well-known to be upregulated in various cancer cells, was successfully developed. By applying gGlu-HMRG to various cancerous cell lines whose GGT activity is upregulated, fast enzymatic reaction of gGlu-HMRG with GGT occurs on the plasma membrane to yield highly fluorescent product HMRG, which led us to establish a novel and highly activatable strategy for sensitive and fast-responding fluorescence imaging of tiny tumors in vivo. In mouse models of disseminated human peritoneal ovarian cancer, activation of gGlu-HMRG occurred within 1 min of topically spraying onto tissue surfaces that are suspected of harboring tumors, creating high signal contrast between the tumor and the background [5]. We believe gGlu-HMRG probe could aid surgeons in detecting tiny cancerous nodules for accurate biopsy and tumor resection, delineating the borders of tumors for complete removal and confirming no residual tumor.

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IL319

5-ALA derivatives in biophotonics: Current state of the art and recent progress

Norbert Lange

University of Geneva, School of Pharmacy, Laboratory of Pharmaceutical Technology, Geneva, Switzerland

The introduction of lipophilic derivatives of the naturally occurring heme precursor 5-aminolevulinic acid (5-ALA) into photomedicine, has led to a true revival of this research area. 5-ALA-mediated photodynamic therapy (PDT) and fluorescence photodetection (FD) of neoplastic disease is probably one of the most selective cancer treatments currently known in oncology. Until today, this method has been assessed experimentally for the treatment of various medical indications. However, the limited local bioavailability of 5-ALA has widely hampered its access to daily clinical practice until today. Although researches became aware of this drawback already early in the development of 5-ALA-mediated PDT, only recently, well established concepts in pharmaceutical science were adapted to this methodology.

Currently, two derivatives of 5-ALA, methylaminolevulinate (MAL) and hexylaminolevulinate (HAL) gained marketing authorization from the regulatory offices in Europe and Australia. MAL is marketed under the trade name Metvix® for the treatment of actinic keratosis (AK) and difficult-to-treat basal cell carcinoma (BCC), HAL has recently been launched under the trade name Hexvix® for the improved diagnosis of superficial bladder cancer in Europe.

Here, I will present the fundamental concepts underlying the use of 5 ALA derivatives in PDT and FD from a chemical, biochemical and pharmaceutical point of view. Despite the tremendous impact of these simple derivatives on biomedical optics, these compounds still suffer from several drawbacks with respect to stability and restricted administration routes. Therefore, new concepts in the research on different 5-ALA derivatives will be discussed.

OC320

Hyperspectral imaging/reflectance spectroscopy of mouse oral tissue exposed to high intensity blue light

Ellen M Bruzell[1], Jon E Dahl[1], Matija Milanic[2], Lise L Randeberg[2]

[1]*Nordic Institute of Dental Materials (NIOM), Oslo, Norway;* [2]*Department of Electronics and Telecommunications, Norwegian University of Science and Technology (NTNU), Trondheim, Norway*

Oral tissues are exposed to optical radiation during various dental treatments and diagnostic methods. However, adverse effects are seldom addressed. Hyperspectral imaging (HSI) and visible near-infrared reflection spectroscopy (VNIRS) were used to observe oral tissue changes in mice exposed to blue light with respect to erythema (erythema index; EI) and pigmentation (melanin index; MI). Pigmented mice (C57BL/6N; $n_{group} = 3$) were exposed on the tongue and the abdominal skin for control or left unexposed. Irradiation was performed with a LED intended for photopolymerisation of dental materials (λ_{peaks}: 409/460 nm) with irradiance ≈ 2 W/cm^2 and radiant exposure ≈ 120 J/cm^2. The hyperspectral measurement was performed by a camera prototype using a cooled EMCCD (1002×204 pixles; λ resolution of 4.0 nm (428-836 nm in the spectral direction); close-up lens (25 mm FOV at a working distance of 83 mm); spatial resolution at sample surface of 25 μm). The camera was scanned past the animals using a motorized translation stage. Reflectance spectra were recorded using a reflectance probe and two fiber optic spectrometers (three measurements per site per animal). MI and EI were calculated based on HSI and VNIRS reflectance spectra, and the values obtained were used to evaluate differences in tissue reflectance between the exposed and control group for each parameter immediately before exposure, immediately after (0.5 h) and ~24 h later. HIS revealed visible changes in tongue tissue scattering observed as "greying" in animals immediately after irradiation and development of condensed tissue after 24 h. Reflectance spectra obtained immediately after exposure confirmed the HSI images by changes in EI and MI of tongue tissue. Hyperspectral imaging is a promising method for assessing tissue changes after irradiation exposure, even in oral tissues of small size animals. The observed tissue damage implies that caution should be exercised when exposing oral tissue to high intensity dental blue light LEDs.

OC321

Optical spectroscopy of the bladder washout fluid to optimize fluorescence cystoscopy with Hexvix®

Carla Martoccia[1], Matthieu Zellweger[1], Gilles Kratzer[1], Blaise Lovisa[1], Patrice Jichlinski[2], Hubert van den Bergh[1], Georges Wagnières[1]

[1]*EPFL Lausanne, Lausanne, Switzerland;* [2]*CHUV Lausanne, Lausanne, Switzerland*

Fluorescence cystoscopy (FC) enhances detection and improves therapeutic outcome of early bladder cancer. During the procedure, water is used to inflate the bladder. It is rapidly mixed with urine. If this Bladder Washout Fluid (BWF) is fluorescent, the FC images are degraded. The main purpose of this study was to explore the fluorescence properties of the BWF to improve image quality during FC. Samples from 15 subjects were collected to assess their spectral properties, as well as the variations induced by pH and temperature changes. Fluorescence lifetime was also measured (excitation wavelength at 320 nm and emission at 420 ± 10 nm). A typical fluorescence spectrum of BWF consists of a main peak (excitation at 347 ± 8 nm and emission at 434 ± 6 nm). In about 40% of cases a secondary peak (excitation range 440-470 nm and emission range 510-540 nm) was observed. Significant inter-patients fluctuation of the fluorescence intensity (SD: 67% of the mean) was observed at room temperature. An increase in temperature (from 35°C to 41°C) induces a 10% decrease in fluorescence intensity of the main peak. The fluorescence intensity was maximal at pH = 7, whereas it decreased by 17 % at pH = 4 and by 12% at pH = 9. In conclusion, changing the temperature or the pH can not be considered as options to decrease the BWF fluorescence intensity in a clinical settings. Fluorescence lifetime measurements showed a bi-exponential decay (τ_1 = 3.2 ns - Rel. Amp. 26 %; τ_2 = 8.7 ns – Rel. Amp. 74 %). These results strongly suggest that 4-pyridoxic acid is the molecule most likely responsible for BWF's fluorescence. Moreover we concluded that the secondary fluorescence peak can be attributed to food and drinks components, such as vitamin B2. The results of this study allow to optimize the spectral design of FC setups.

OC322

Measurement of tissue oxygenation *in vivo* by time-resolved luminescence spectroscopy of Ru(Phen), a poorly photosensitizing probe

Veronika Huntosova, Sandrine Gay, Patrycja Nowak-Sliwinska, Georges Wagnières

EPFL Lausanne, Lausanne, Switzerland

Measuring tissue oxygenation in vivo is of interest for numerous fundamental and applied studies. One minimally-invasive approach to assess the oxygen partial pressure (pO_2) in tissue is to measure the oxygen-dependent luminescence lifetime of molecular probes. The relation between the tissue pO_2 and this lifetime is governed by the Stern-Volmer equation. Unfortunately, virtually all oxygen-sensitive probes based on this principle induce some degree of phototoxicity. For that reason we studied the oxygen sensitivity and phototoxicity of dichlorotris (1,10-phenantroline)-Ruthenium(II) Hydrate (Ru(Phen)) using a dedicated optical fiber-based, time-resolved spectrometer in the chicken embryo chorioallantoic membrane (CAM). We demonstrated that, after intravenous injection, Ru(Phen)'s

luminescence lifetime presents an easily detectable pO_2 dependence at a low drug dose (1 mg/kg) and low fluence (120 mJ/cm^2 at 470 nm). The phototoxic threshold was found to be at 10 J/cm^2 with the same wavelength and drug dose, i.e. about two orders of magnitude higher than the fluence necessary to perform a pO_2 measurement. Finally, an illustrative application of this pO2 measurement approach in a hypoxic tumor environment is presented.

OC323

Theranosomes: Translating Cell-Released Vesicles into Smart Nanovectors

Amanda K. A. Silva[1], Stéphanie Bonneau[2], Florence Gazeau[1], Claire Wilhelm[1]

[1]*Université Paris Diederot - Laboratoire MSC, Paris, France;* [2]*Université Pierre et Marie Curie - Laboratoire Jean Perrin, Paris, France*

Cell-released vesicles are natural carriers that circulate in body fluids and transport biological agents to distal cells. As nature uses vesicles in cell communication to promote tumor progression, we propose to harness their unique properties and exploit these biogenic carriers as Trojan horses to deliver therapeutic payloads to cancer cells. In a theranostic approach, cell-released vesicles were engineered by a top-down procedure from precursor cells, previously loaded with a photosensitizer and magnetic nanoparticles. The double exogenous cargo provided vesicles with magnetic and optical responsiveness allowing therapeutic and imaging functions. This new class of cell-derived smart nanovectors was named "theranosomes". Theranosomes enabled efficient photodynamic tumor therapy in a murine cancer model in vivo. Moreover the distribution of this biogenic vector could be monitored by dual-mode imaging, combining fluorescence and MRI. This study reports the first success in translating a cell communication mediator into a smart theranostic nanovector.

OC325

Improvement of the selectivity of Photodynamic Therapy (PDT): the «Photodynamic Molecular Beacons ».

Aurélie Stallivieri[1], Marc Verhille[1], Hamanou Bénachour[2], Philippe Arnoux[1], Muriel Barberi-Heyob[2], Xavier Allonas[3], Francis Baros[1], Jérôme Devy[4], Régis Vanderesse[5], Céline Frochot[1]

[1]*LRGP UMR 7274, University of Lorraine, CNRS, Nancy, France;* [2]*CRAN UMR 7039, University of Lorraine, CNRS, Vandœuvre-lès-Nancy, France;* [3]*LPIM EA 4567, University of Haute Alsace, CNRS, Mulhouse, France;* [4]*SiRMa UMR 6237, University of Reims Champagne-Ardenne, CNRS, Reims, France;* [5]*LCPM FRE 3564, University of Lorraine, CNRS, Nancy, France*

One limitation of photodynamic therapy (PDT) is the low selectivity of photosensitizers to tumour tissue or neovascularization. The synthesis of photosensitizers coupled with substrate specific of the membrane receptors becomes a center of interest. Another strategy is to produce reactive oxygen species specifically at the tumor site. It is this approach that fits our research. A promising approach is to use the activity of enzymatic cleavage of biomarkers overexpressed in tumour areas. Different enzymes such as matrix metalloproteinases (MMPs) are overexpressed in tumour development zones. Among these MMPs, gelatinases (MMP-2 and MMP-9) and its activator MMP-14 are known to play a key role in tumour angiogenesis [1, 2, 3] and the growth of many cancers such as glioblastoma multiforme (GBM), an aggressive malignant tumor of the brain [4, 5]. We describe the synthesis, photophysical properties and enzymatic action of different photodynamic molecular beacons (PMB), composed of a chlorin as a photosensitizer, and a quencher linked together by a peptide substrate of gelatinase or MMP-14. [6]

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OC326

Homology Modeling of Human γ-Butyric Acid Transporters and the Binding of Pro-drugs 5-Aminolevulinic Acid and Methyl Aminolevulinic Acid used in Photodynamic Therapy

Yan Baglo[1], Mari Gabrielsen[2], Ingebrigt Sylte[2], Odrun Arna Gederaas[1]

[1]*Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway;* [2]*Faculty of Health Sciences, University of Tromsø, Tromsø, Norway*

Photodynamic therapy (PDT) is a safe and effective method currently used in the treatment of skin cancer. In ALA-based PDT, 5-aminolevulinic acid (ALA), or ALA esters, are used as pro-drugs to induce the formation of the potent photosensitizer protoporphyrin IX (PpIX). Activation of PpIX by light causes the formation of reactive oxygen species (ROS) and toxic responses.
Studies have indicated that ALA and its methyl ester (MAL) are taken up into the cells via γ-butyric acid (GABA) transporters (GATs). Uptake via GATs into peripheral sensory nerve endings may also account for one of the few adverse side effects of ALA-based PDT, namely pain. In the present study, homology models of the four human GAT subtypes were constructed using three x-ray crystal structures of the homologous leucine transporter (LeuT) as templates. Binding of the native substrate GABA and the possible substrates ALA and MAL was investigated by molecular docking of the ligands into the central putative substrate binding sites in the outward-occluded GAT models. Electrostatic potentials (ESPs) of the putative substrate translocation pathway of each subtype were calculated using the outward-open and inward-open homology models.
Our results suggested that ALA is a substrate of all four GATs and that MAL is a substrate of GAT-2, GAT-3 and BGT-1. The ESP calculations indicated that differences likely exist in the entry pathway of the transporters (i.e. in outward-open conformations). Such differences may be exploited for development of inhibitors that selectively target specific GAT subtypes and the homology models may hence provide tools for design of therapeutic inhibitors that can be used to reduce ALA-induced pain.

OC327

Singlet oxygen photosensitization by the genetically-encoded tag miniSOG

Rubén Ruiz-González[1], Aitziber L. Cortajarena[2], Sara H. Mejias[2], Montserrat Agut[1], Santi Nonell[1], Cristina Flors[2]

[1]*Institut Químic de Sarrià, Barcelona, Spain;* [2]*Madrid Institute for Advanced Studies in Nanoscience (IMDEA Nanociencia), Madrid, Spain*

Genetically-encodable fluorescent tags that are able to generate reactive oxygen species upon light irradiation are of great interest in applications such as chromophore-assisted laser inactivation (CALI), optogenetics and photodynamic therapy. Specifically, fluorescent proteins that photosensitize singlet oxygen ($^1O_2$) are of special relevance for correlative light and electron microscopy

(EM), as $^1O_2$ locally photooxidizes diaminobenzidine to form an osmiophilic precipitate that gives contrast in EM.
We have previously shown that some variants from the green fluorescent protein (GFP) family are able to photosensitize $^1O_2$ with very low efficiency (Φ_Δ= 0.004) [1-3]. Recently, efforts to produce genetically-encodable tags that generate $^1O_2$ more efficiently have turned to the engineering of flavin mononucleotide (FMN)-binding proteins, since FMN is a good $^1O_2$ photosensitizer (Φ_Δ = 0.51). MiniSOG (for "mini Singlet Oxygen Generator") is a 106-aminoacid flavoprotein derived from phototropin 2 that is expected to revolutionize correlative light- and electron microscopy [4].
Using time-resolved detection of $^1O_2$ phosphorescence at 1275 nm, along with other laser spectroscopic and biochemical techniques, we have unravelled the complex photochemistry of miniSOG [5]. Our findings show that miniSOG photosensitizes $^1O_2$ with a 15-fold lower efficiency than initially thought, and that is able to undergo photoinduced electron-transfer reactions. In addition, we find that cumulative irradiation of miniSOG increases its photosensitization ability 10-fold due to a photoinduced transformation of the protein. This may be the reason why miniSOG outperforms every other fluorescent protein reported to date as $^1O_2$ generator.

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OC328

Phthalocyanine-Peptide Conjugates: Receptor-Targeting Bifunctional Agents for Imaging and Photodynamic Therapy

Johan E. van Lier, Elena Ranyuk, Nicole Cauchon, Brigitte Guerin

University of Sherbrooke, Sherbrooke, QC, Canada

The synthesis of a series of new zinc phthalocyanine-peptide conjugates targeting the gastrin-releasing peptide (GRP) and integrin receptors is reported. Two alternative synthetic methods based on Sonogashira cross-coupling of an iodinated zinc phthalocyanine with acetylenic bombesin or arginine–glycine–aspartic acid (RGD) derivatives, either in solution or on solid phase, are presented. The water-soluble conjugates were screened for their photodynamic efficacy against several cancer cell lines expressing different levels of GRP and integrin receptors, and their intracellular localization was evaluated via confocal fluorescence microscopy. Variations in photocytotoxicity between the conjugates correlate to differences in hydrophobicity as well as receptor-mediated cell uptake. In the case of the phthalocyanine-bombesin conjugate, competition experiments confirm the involvement of the GRP receptor in both the phototherapeutic activity as well as intracellular localization. These findings warrant further *in vivo* studies to evaluate the potential of this conjugate as photosensitizer for photodynamic therapy (PDT) of cancers over-expressing the GRP receptor.

OC329

Biocompatible nanoparticles doped with arylcyanoporphyrazine chromophores: design, in vitro and in vivo behavior

Irina V. Balalaeva[1], Natalia Yu. Shilyagina[1], Larisa G. Klapshina[2], Svetlana A. Lermontova[2], Alexander V. Yakimansky[3], Marina V. Shirmanova[4], Elena V. Zagaynova[4]

[1]*State University of Nizhny Novgorod, Nizhny Novgorod, Russian Federation;* [2]*Razuvaev Institute of Organometallic Chemistry of RAS, Nizhny Novgorod, Russian Federation;* [3]*Institute of Macromolecular Compounds of RAS, Saint Petersburg, Russian Federation;* [4]*Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russian Federation*

Tetrapyrrolic macrocycles and their metal complexes occupy a central place among organic compounds applied in the fields of neoplastic tissue detection and photodynamic therapy. We have studied a series of novel fluorescent tetra(aryl)tetra(cyano)-porphyrazine free bases and their ytterbium complexes, characterized by strong extinction and luminescence in the red spectral region. To ensure biocompatibility the chromophores were incorporated into nanoparticles, based on non-toxic water-soluble polymers such as polyethylene glycol, methylcellulose, sodium alginate, and polyimide-graft-(polymethacrylic acid) polymeric brushes. Obtained chromophore-doped nanoparticles are low-toxic in dark conditions that allow employing them for biomedical applications. Rapid cellular uptake by clathrin-independent endocytosis and intacellular accumulation of the nanoparticle-incorporated chromophores has been shown.
The arylcyanoporphyrazine free bases are able to generate singlet oxygen at light exposure, and as a result, they cause photoinduced damage of the chromophore-preincubated cells (photodynamic effect).
Phototoxicity of the porphyrazine free bases is comparable to clinically approved chlorine and phthalocyanine drugs. Ytterbium complexing to macrocycle results in shift in contribution of excited state relaxation paths and almost withdraw the photodynamic activity.
Among the most significant properties responsible for chromophore efficiency for *in vivo* diagnosis/treatment there is an ability to be selectively accumulated in site of alteration, such as tumor or inflammation. *In vivo* study has demonstrated that i.v. injection of the porphirazine-doped nanoparticles to tumor-bearing mice allow clear visualization of tumors by methods of whole-body fluorescent imaging. Quantification of the fluorescence in the tumor area provided an opportunity to define tumor uptake and retention kinetics. High contrast in the porphirazine concentration between tumor sites and surrounding normal tissues has been confirmed by microscopy studies. The *in vivo* photodynamic activity of arylcyanoporphyrazines and their metal complexes is to be estimate.

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OC330

A Fluorinated Bacteriochlorin as a Photostable Photodynamic Therapy Agent: From Synthesis to In Vivo Studies

Janusz M. Dąbrowski[1], Mariette M. Pereira[2], Luís B. Rocha[3], Martyna Krzykawska-Serda[4], Krystyna Urbańska[4], Grażyna Stochel[1], Luís G. Arnaut[2,3]

[1]*Faculty of Chemistry, Jagiellonian University, Kraków, Poland;* [2]*Chemistry Department, University of Coimbra, Coimbra, Portugal;* [3]*Luzitin SA, Ed. Bluepharma, Coimbra, Portugal;* [4]*Faculty of Biochemistry, Biophysics and Biotechnology, Kraków, Poland*

Photodynamic therapy requires the combination of a photosensitizer, light and oxygen to generate reactive oxygen

species (ROS) that destroy the diseased tissue. The directionality of light, the eventual affinity of photosensitizers towards tumors and the short diffusion radius of the ROS minimize the damage to healthy tissues, and make of PDT a very well tolerated therapy.

The search for "ideal photosensitizer" focuses on bacteriochlorin derivatives because they meet most of desired properties, although earlier studies projected the idea that bacteriochlorins were too labile for PDT. The development of the family of halogenated photostable bacteriochlorins led to a chlorinated sulfonamide bacteriochlorin (Cl_2BEt) that delayed the growth of S91 melanoma tumors subcutaneously implanted in DBA mice by 44 days with respect to control.

We now report a new and most efficient PDT photosensitizer of this family: 5,10,15,20-tetrakis(2,6-difluoro-3-N-methyl-sulfamoylphenyl)bacteriochlorin (F_2BMet, LUZ11). It was synthesized using a solvent-free method, and fully characterized. This bacteriochlorin closely approaches the properties of an "ideal photosensitizer": simple synthesis yielding a pure compound, molar absorption coefficient above 100,000 M^{-1} cm^{-1} in the phototerapeutic window, controlled photostability, measurable fluorescence, *n*-octanol:water partition coefficient of 80, very low toxicity in the dark, and quantum yield of reactive oxygen species ca. 0.6. We have also evaluated its cellular uptake, cytotoxicity and photodynamic activity against various tumor cells (A549, S91-I3, CT26, PC-3, HT-29). Our cellular studies revealed intracellular localization in the endoplasmic reticulum, generation of radicals in the cells and changes in the mechanism of cell death as a function of the light dose. The pharmacokinetics and biodistribution were studied in DBA/2 mice bearing S91 melanoma tumors. Exploratory PDT was performed 15 min., 24 h and 72 h after i.v. administration, and led to the complete disappearance of tumors for approximately 2 months and some of the animals treated with the 15 min. protocol were completely cured.

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OC331

Photochemical internalization of cancer stem cell-targeting therapeutics

Pål Kristian Selbo[1,2], Monica Bostad[1], Marius Strømbo Eng[1], Jagdip Kaur[1], Anders Høgset[2], Kristian Berg[1]

[1]*Norwegian Radium Hospital, Oslo, Norway;* [2]*PCI Biotech, Oslo, Norway*

Cancer stem cells (CSCs) have acquired characteristics associated with normal stem cells, i.e. self renewal and capacity to differentiate and give rise to non-tumorigenic progeny. CSC seems to be orchestrated by the microenvironment of the tumor, e.g. hypoxia has been demonstrated to induce over-expression of CSC markers such as ABCG2, CD133 and CD44. CSCs have also an efficient machinery to cope with reactive oxygen species and are hence thought to be resistant against radiotherapy and some chemotherapies. The CSC concept may have significant implications for the outcome photodynamic therapy (PDT), as CSC must be killed in order to obtain complete response and long-term disease-free survival. Consequently, it is of great importance to establish drugs and/or therapies that specifically target and eliminate CSC.

Photochemical internalization (PCI) is an efficient and specific drug and gene delivery technology established in our lab for the light-induced endosomal or lysosomal membrane rupture and escape of molecules sequestered in these organelles. PCI of the chemotherapeutic drug bleomycin is currently in a Phase II clinical trial at 5 different centres in Europe.

Previously we have demonstrated that PCI-photosensitizers are not substrates of the efflux pump and the CSC marker ABCG2/BCRP.

In this presentation we will present data showing that Amphinex-PCI-based targeting of CSC markers including CD133, CD44, CSPG4/NG2, CD271 and CD90 is highly specific and efficient in a number of different cancer types. In addition, *in vitro* PCI-based purging of a <2% CD133-expressing sarcoma population results in a decreased tumor-initiation of surviving cells in NOD-SCID IL2Rgamma^{null} mice.

PCI of therapeutics targeting different CSC markers provides high selectivity and potent cytotoxicity establishing PCI as a potential rational for elimination of CSC.

OC332

Monomeric pheophorbide(a)-containing poly(ethyleneglycol-b-ε-caprolactone) micelles for photodynamic therapy: mechanisms of cellular internalisation

Rachid Kerdous[1], Laure Gibot[2], Anne-Françoise Mingotaud[3], Patricia Vicendo[3], Stéphanie Bonneau[1]

[1]*Université Pierre et Marie Curie, Paris, France;* [2]*Université Toulouse 3, Toulouse, France;* [3]*CNRS - Université Toulouse 3, Toulouse, France*

Poly(ethyleneglycol-b-ε-caprolactone) micelles constitute a powerful and promising drug delivery system due to their capability to enhance the solubility, the pharmacokinetics properties and the biodistribution of drugs. Here, we used such nanoparticles loaded with pheophorbide(a) to internalize the photosensitizer within MCF-7 cells. We show that the incorporation is increase by 1.5 by that nanocarriers comparing with pheophorbide(a) alone, while the endocytosis of the nanoparticles themselves is very low. Moreover, the kinetics of cellular incorporation is clearly different in both cases.

To decipher the mechanisms involved, complementary experiments have been performed with models of membranes. Naïve unilamellar vesicles have been mixed with pheophorbide(a)-preloaded nanoparticles. The kinetics of the transfer of the photosensitizer to membrane have been recorded and analyzed.

Our results clearly demonstrate that the transfer occurs by direct contact between the membranes and the nanoparticles, the pheophorbide(a) being exchange by this collisional process.

OC333

Impact of photodynamic stress on intracellular trafficking and viscosity

Kelly Aubertin[1], Stéphanie Bonneau[2], Amanda K.A. Andriola[1], François Gallet[1], Claire Wilhelm[1]

[1]*Université Paris Diderot - Laboratoire MSC, Paris, France;* [2]*Université Pierre et Marie Curie - Laboratoire Jean Perrin, Paris, France*

The intracellular microenvironment is essential for the efficiency of photo-induced therapies, as short-lived reactive oxygen species generated must diffuse through their intracellular surrounding medium to reach their cellular target. Here, by combining measurements of local cytoplasmic viscosity and active trafficking, we found that phototherapy induced a only slight increase in viscosity but a massive decrease in diffusion. These effects are the signature of a return to thermodynamic equilibrium of the system after photo-activation and correlated with depolymerization of the microtubule network, as shown in a reconstituted system. These mechanical measurements were performed with two intracellular photosensitizing chlorins having similar quantum yield of singlet oxygen production but different intracellular localizations (cytoplasmic for m-THPC, endosomal for TPCS2a). This two agents demonstrated different intracellular impact.

OC334

In vitro photophysical and photobiological properties of Ce6-based dendrimer nanoparticles

Estelle Bastien[1], Raphael Schneider[2], Lina Bezdetnaya[1], Beate Röder[3], Steffen Hackbarth[3], Annegret Preuß[3], Jordane Jasniewski[4], Vadzim Reshetov[1], Henri-Pierre Lassalle[1]

[1]*Centre de recherche en automatique de Nancy, Université de Lorraine, CNRS UMR 7039, Institut de Cancérologie de Lorraine, Nancy, France;* [2]*Laboratoire Réactions et Génie des Procédés, Ecole National Supérieure des Industries Chimiques, Université de Lorraine, Nancy, France;* [3]*Institut of physic, Humboldt University, Berlin, Germany;* [4]*Laboratoire d'ingénierie des biomolécules, Université de Lorraine, Nancy, France*

Context: The photosensitizers vectorization enables better tumor localization and therefore a better tumoricidal PDT efficacy. Dendrimers are hyperbranched macromolecules that are considered as an attractive class of biocompatible nanovectors for drug targeting in tissues. In this study we report a novel PDT drug-carrier system, composed of chlorin e6 (Ce6) grafted to the periphery groups of poly(amido amine) dendrimers (PAMAM).

Materials & Methods: The size and zeta potential of our nanoparticles were determined by dynamic light scattering (DLS). The nanoparticle singlet oxygen generation in pharynx carcinoma (FaDu) cells was assessed by luminescence at 1268 nm. Phototoxicity was evaluated by MTT assay. Uptake was assessed by chemical extraction associated with fluorescence spectroscopy measurements. Finally, the uptake mechanism was determined by the use of endocytosis specific inhibitors.

Results: Ce6-dendrimers nanoparticles displayed a 40 times greater uptake along with a 25 fold higher photocytotoxic activity in FaDu cells compared to free Ce6. Also, a much longer singlet oxygen lifetime was demonstrated for Ce6-dendrimers compared to Ce6. Cellular incorporation of nanoparticles was completely inhibited at 4°C, demonstrating that uptake was mediated via endocytosis. Further investigations using specific inhibitors revealed that incorporation was partially mediated by macropinocytosis pathway.

Conclusion: Dendrimeric based nanoparticles showed a good photodynamic efficacy towards cancer cells *in vitro*, suggesting them as a promising vehicle for PDT. In the future we shall test dendrimer-based nanoparticles *in vivo*, in pre-clinical models.

OC335

Effect of salts on merocyanine 540 aggregation and inactivation of *Staphylococcus aureus* and *Pseudomonas aeruginosa* photosensitized by this dye

Tatiana Shmigol[1], Elena Sysolyatina[2], Elena Nagurskaya[2], Svetlana Ermolaeva[2], Natalia Vlasova[1], Alexander Potapenko[1]

[1]*N.I. Pirogov Russian National Research Medical University, Moscow, Russian Federation;* [2]*Gamaleya Research Institute of Epidemiology and Microbiology, Ministry of Health and Social Development of Russian Federation, Moscow, Russian Federation*

Merocyanine 540 (MC540) is an anionic photosensitizer used in antimicrobial photodynamic therapy (aPDT). In aqueous salt solutions MC540 can exist in several aggregative states. In water, MC540 is dissociated, the addition of a salt leads to the formation of large aggregates which can be detected by method of resonant light scattering (RLS). We have established the existence of the critical aggregation concentration (CAC) of salt above which the solution begins the formation of large aggregates. We have proved that in solutions of monovalent cations product of concentrations of MC540 by CAC is a constant, which is the ion product (Ksp) of ionized forms of the dye. Knowing the Ksp we can calculate of the proportion of aggregated MC540 molecules.

We have shown that aggregates of MC540 are more photolabile than ionized forms of MC540. It is well known that singlet oxygen can generate only MC540 monomers. Increase in rate of photolysis may indicate switching of reaction mechanism towards free radical reactions. Switching of photochemical reactions was confirmed by addition of antioxidants. In the present work we investigated the influence of aggregative state of 25 μM MC540 on the effectiveness of photodynamic inactivation of *S. aureus* and *P. aeruginosa* in water and 0.25 M aqueous NaCl. Calculations show that used NaCl and MC540 concentrations lead to aggregation of dye molecules in amount not less than 75%. These results show that aggregation of MC540 in presence of salt essentially increases the efficiency of photosensitized MC540 inactivation of *S. aureus* and *P. aeruginosa* (25 times and 15 times) respectively. We have also shown that further increase in time of incubation of MC540 with bacteria in aqueous salt solutions from 10 to 40 minutes, decreases the efficiency of aPDT. This indicates that free radical attack at bacterial cells occurs from outside. In case of short-term incubation of cells with aggregates of MC540 they do not have time to disaggregate and attack bacteria with free radicals, and in case of prolonged incubation, occurs disaggregation leading to reduction of production of radicals and decrease in cellular damage.

OC336

Massive apoptosis without cell detachment induced by a synergistic-PDT treatment

Pilar Acedo, Ángeles Villanueva, Magdalena Cañete, Juan Carlos Stockert

Autonomous University of Madrid, Madrid, Spain

Combinations of various therapeutic modalities with non-overlapping toxicities are the commonly used strategies to improve the therapeutic index of treatments in modern oncology. There have been few studies examining the effectiveness of PDT with standard antitumor regimens in order to improve the usefulness of PDT. One incoming approach to promote this idea involves the development of novel protocols combining two PSs that synergistically enhance the cytotoxic effects of PDT. In the present study, we attempt to open a new way in PDT-treatments by using simultaneously two photosensitizers: Zinc(II) phthalocyanine (ZnPc) and the cationic porphyrin meso-tetrakis (4-N-methylpyridyl)porphine (T4MPyP). This combined treatment produced an enhanced lethal effect relative to treatments with single PSs, in three different human cell lines: cervix adenocarcinoma (HeLa), breast adenocarcinoma (MCF-7) and keratinocytes (HaCaT). Both, apoptotic and necrotic cell death mechanisms can occur in HeLa cells depending on the experimental protocol (1 h incubation with both PSs + 2.4 or 3.6 J/cm^2 light dose). The goal of this research was to discover the induction of a massive apoptosis without loss of cell-substrate adhesion (typical of the apoptotic process). Different alterations in the distribution and organization of cytoskeletal elements (microtubules and actin microfilaments), as well as, vinculin and focal adhesion kinase, have been analyzed by immunostaining and time-lapse video microscopy. Taking into account the relevant role of cytoskeletal components in cancer cells invasion and metastasis, these structures constitute a basic and important target of PDT. The reduced doses used for combination therapies, could result in lower side effects, improved treatments and better outcomes than monotherapy, which may be beneficial in clinical PDT.

OC337

Photodynamic therapy with conventional and PEGylated liposomal formulations of temoporfin: comparison of treatment efficacy and distribution characteristics in vivo

Vadzim Reshetov[1,8], Henri-Pierre Lassalle[2], Aurélie François[2,3], Dominique Dumas[4], Sebastien Hupont[4], Susanna Gräfe[5], Vasco Filipe[6,7], Wim Jiskoot[6,7], François Guillemin[2,3], Vladimir Zorin[1], Lina Bezdetnaya[2,3]

[1]*Laboratory of Biophysics and Biotechnology, Belarusian State University, Minsk, Belarus;* [2]*Université de Lorraine, Centre de Recherche en Automatique de Nancy (CRAN), Nancy, France;* [3]*Lorraine Cancer Institute, Nancy, France;* [4]*Université de Lorraine, Plate forme d'Imagerie et de Biophysique cellulaire PTIBC IBISA NANCY, Nancy, France;* [5]*Biolitec GmbH, Research & Development, Jena, Germany;* [6]*Division of Drug Delivery Technology, Leiden, Netherlands;* [7]*Amsterdam Center for Drug Research, Leiden University, Leiden, Netherlands;* [8]*RUE "Belmedpreparaty", Minsk, Belarus*

A major challenge in the application of nanoparticle drug delivery system is understanding the properties that influence their *in vivo* behavior and therapeutic performance of the anticancer drug. The effect of liposomal formulation as an example of widely-used delivery system on all aspects of the drug delivery process should be considered when optimizing the treatment with liposomal drugs. We present a comparison of conventional (Foslip®) and PEGylated (Fospeg®) liposomal formulations of temoporfin (meta-tetra(hydroxyphenyl)chlorin) in tumor-grafted mouse model. Foslip® and Fospeg® pharmacokinetics, drug release, liposome stability, tumor uptake and intratumoral distribution are evaluated, and their influence on the efficacy of the treatment at different light-drug intervals is discussed. Multiphoton fluorescence macroscopy imaging was used to visualize the *in vivo* intratumoral distribution of the photosensitizer. The combination of EPR-based tumor accumulation, stability in the circulation and release properties leads to a higher efficacy of the treatment with Fospeg® compared to Foslip®. A significant advantage of Fospeg® lies in a major decrease in the light-drug interval, while preserving the treatment efficacy.

OC338

Synthesis and characterization of photosensitizers-conjugated magnetic nanoparticles - Potential anticancer agents for application in Photodynamic Therapy.

Vincent Sol, Jean-Pierre Mbakidi, Tan-Sothea Ouk, Robert Granet, Pierre Krausz

Laboratoire de Chimie des Substances Naturelles, Université de Limoges, Faculté des sciences, Limoges, France

Photodynamic therapy (PDT) is a non –invasive cancer treatment based on the administration of photosensitizer, followed by light irradiation. To improve the efficiency of photosensitizer, we have developed the synthesis of magnetic nanoparticles porphyrin by click chemistry or amidation reactions. Indeed, nanoparticles penetrate easily the cancer cell by a process of passive endocytosis (EPR effect). Such magnetic nanoparticles carrying a photosensitive drug should help to develop molecular platforms to double efficiency by combining the lethal effect photocytotoxic drugs with magnetic properties of the carrier (thermotherapy). We have developed synthesis of iron oxide nanoparticles stabilized in aqueous medium by the modified dextran. Glucosylated, cationic, sulfonated porphyrins and polyethylene imine (PEI) were covalently attached to these nanoparticles by triazole ring. Another nanoparticle bearing chlorine e6-PEI directly by the use of epichlorhydrin were so synthetized. All particles have been detected by various spectroscopic (UV-visible, fluorescence, IR, ^{1}H NMR) and physicochemical (Mass, TEM, magnetometry, ATG) methods. Photocytotoxic activity of these porphyrins-nanoparticles derivatives was tested on keratinocyte cell line (HaCaT cell lines) and compared to Photofrin II®.

IL339

Metallic Nanoparticles for use in Biomedical Analysis

Duncan Graham

University of Strathclyde, Glasgow, United Kingdom

Metallic nanoparticles offer many opportunities in terms of detection including light scattering, surface plasmon resonance and surface enhanced resonance scattering (SERS). We are interested in the optical properties of metal nanoparticles and their potential application in a range of different biological studies. We can make use of the optical properties of nanoparticles in two ways.

1) The nanoparticle can act as an extrinsic label for a specific biomolecular target in the same way as a fluorescent label is used. The advantage of using the nanoparticle is its optical brightness (typically several orders of magnitude more than fluorophores) and the lack of background vibrational signals. Functionalisation of the nanoparticle with a specific targeting species such as an antibody or peptide aptamer allows this approach to be used in a wide range of studies including cell, tissue and in vivo analysis.
2) Nanoparticles can be designed to contain a specific recognition probe designed to cause a change in the aggregation status of the nanoparticles resulting in a discernible optical change when it interacts with its biomolecular target. This allows separation free analysis of specific biomolecular interactions and can be applied to a range of different probe/target interactions such as DNA-DNA, peptide-protein and sugar-protein.

We have been making use of nanoparticles in both of these approaches in conjunction with SERS which is an advanced vibrational spectroscopy. To demonstrate the applicability of the two different approaches examples will be given on the use of nanoparticles for cell imaging in two and three-dimensions, imaging of nanoparticles at centimetre depths through tissue and also their ability to report on biological molecules in vitro and in vivo.

IL340

Photodynamic therapy-based combinations made more effective with multi-inhibitor nanoconstructs

Tayyaba Hasan

Wellman Center for Photomedicine, Harvard Medical School, Massachusetts General Hospital, Boston, MA, United States

Cancer treatment with curative intent requires multimodality therapeutic strategies. There is evidence that treatments targeting key molecular pathways that mechanistically synergize with PDT will prove most effective. We have been developing multi-compartmental constructs capable of delivering the photosensitizer along with inhibitors simultaneously so as to allow optimal mechanistic interaction. The photochemical process involved in PDT potentially also aids endosomal escape for these constructs so that the inhibitors reach their intracellular targets more efficiently. Tumor heterogeneity both at the macroscopic and the microscopic levels requires such interactive multi-targeted approaches. This presentation will discuss the targets appropriate for interaction with PDT, and will present results from pre-clinical therapy experiments with implications for clinical studies.

OC341

Nanosensors and nanoprobes for localised intracellular pH measurements within acidic organelles of mammalian cells

Maria J. Marin[1], Francisco Galindo[2], Paul Thomas[3], Tom Willeman[4], David A. Russell[1]

[1]*School of Chemistry, University of East Anglia, Norwich, United Kingdom;* [2]*Department of Inorganic and Organic Chemistry, University Jaume I, Castellon de la Plana, Spain;* [3]*School of Biological Sciences, University of East Anglia, Norwich, United Kingdom;* [4]*Norwich Medical School, University of East Anglia, Norwich, United Kingdom*

Protons play an important role in the optimal functioning of the different organelles that constitute a eukaryotic cell.[1] The application of fluorescent photoinduced electron transfer (PET) molecular probes for the imaging of intracellular proton concentration in acidic organelles has been proven to be successful.[2] Our research pursues two aims: 1) the synthesis of a novel PET based pH nanoprobe and its biological application for the signalling of acidic organelles in mammalian cells;[3] and 2) the synthesis of a ratiometric PET based pH nanosensor that enables the pH of individual regions within cells to be determined.[4]

A fluorescent ligand sensitive to pH that incorporates a thiolated moiety was synthesised and used to stabilise gold nanoparticles yielding a PET based pH nanoprobe. Following the classic PET behaviour, the fluorescence emission of the nanoprobe was quenched in alkaline conditions and enhanced in an acidic environment. The nanoprobe was used for the intracellular imaging of acidic environments within Chinese hamster ovary (CHO) cells by confocal laser scanning microscopy (CLSM). The internalisation of the nanoprobe by the cells was confirmed by confocal fluorescence images and also by recording fluorescence emission spectra of the nanoprobe from within the cells. Co-localisation experiments using a marker of acidic organelles and a marker of autophagosomes confirmed that the PET based nanoprobe acts as a marker of acidic organelles and autophagosomes within mammalian cells.

The PET based pH ligand and a ratiometric ligand were used to functionalise the surface of gold nanoparticles yielding a ratiometric PET based pH nanosensor. The variation of the fluorescence signals of the nanosensor with pH enabled ratiometric measurements of pH between 3.5 and 6.5. The ratiometric pH nanosensor was used for intracellular imaging of CHO cells using CLSM. The intracellular emission spectrum confirmed the internalisation of the nanosensor. Co-localisation experiments using a selective probe for acidic organelles suggested that the ratiometric pH nanosensor accumulated in the acidic organelles within the CHO cells. The pH nanosensor accumulated within the cells enabled the pH of specific regions to be calculated. For all the pH measurements, the mean value was 5.2 ± 0.7 which is in agreement with the accepted pH for acidic organelles.[1]

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OC342

Polyaniline- Based Nano-Material as an Optical Sensor

Wessam Saleh Omara[1], Shaker Ebrahim[2], Wegdan Ramadan[3], Moataz Soliman[2], Rehab Amin[1]

[1]*National Institute of Laser Enhanced Sciences, Cairo University, Cairo, Egypt;* [2]*Institute of Graduate Studies and Research, Alexandria University, Alexandria, Egypt;* [3]*Faculty of Science, Alexandria University, Alexandria, Egypt*

Nanotechnology attracts considerable attention and promising future for interdisciplinary and applied science due to the small size and precise characters of the nano-materials. The aim of the current study was to develop an efficient formaldehyde sensor. Polyaniline nanoparticle and gold-polyaniline nanocomposites were prepared, characterized and applied independently as formaldehyde sensors. Formaldehyde sensing was evaluated either optically using microplate assay or electrochemically using potentiostat. Results showed that; polyaniline nanoparticle is an efficient sensor, whereas it can detect law concentrations of formaldehyde starting from $3x10^{-5}$ ppm. On the other hand, gold-polyaniline nanocomposites showed no significant sensitivity for the detection of low concentrations of formaldehyde. Therefore, polyaniline nanoparticle could be used as a rapid, cheap, stable and sensitive formaldehyde sensor.

OC343

Influence of Particles on the Performance of Sunscreens

Bernd Herzog

BASF Grenzach GmbH, Grenzach-Wyhlen, Germany

UV absorbers are the active ingredients responsible for the protective effect of sunscreens against the damaging UV radiation of the sun. Most of these ingredients are soluble either in water or in cosmetic oils, but inorganic and organic particulate UV filters acting via absorption and scattering are also very common. Particles in the sub-micron size-range present in sunscreen formulations are expected to increase the pass length of the radiation in the sunscreen film on the skin due to their scattering effect. Hence, the efficacy of oil- or water-soluble UV absorbers present in the same film is expected to be enhanced. This phenomenon is investigated with non-absorbing and with UV-absorbing particles in model systems. Particulate UV filters, although mainly acting by absorption rather than scattering, show an efficacy increasing effect as well as non-absorbing particles.

IL344

NIR-light triggered delivery of macromolecules into the cytosol

Susana Carregal-Romero, Markus Ochs, Joanna Rejman, Wolfgang J. Parak

Department of Physics and WZMW, Philipps Universität Marburg, Marburg, Germany

Light-responsive microcapsules constructed by layer-by-layer self-assembly are used as microcarriers to deliver different macromolecules inside cells.[1,2] The microcapsules carry the macromolecules as cargo in their cavity, while their walls are modified with agglomerated gold nanoparticles. Microcapsules are incorporated by living cells and are then located in lysosomal compartments. Controlled release of the encapsulated material from the interior of the capsule to the cytosol is possible upon NIR-light irradiation. This is based on local heating of the gold nanoparticles upon NIR light and disruption of the capsule wall, what results on release of encapsulated materials. We illustrate several key advances in controlled release induced by light. First, we demonstrate that capsules can be opened individually, which allows for sequentially releasing cargo from different capsules within one single cell. Second, by using a pH-indicator as cargo the claim of release from the acidic lysosomal compartments to the neutral cytosol is experimentally demonstrated. Third, green fluorescent protein (GFP) and mRNA encoding GFP are released to the cytosol while retaining its functionality. This demonstrates that functional molecules can be released without destruction by the local heating. Moreover, it is possible to study the kinetics of biological reactions triggered upon the delivery of active molecules into the cytosol.

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IL345

Upconverting Fluorescent Nanoparticles: Property and Biological Applications

Muthu Kumara Gnanasammandhan, Yong Zhang

Department of Bioengineering, Faculty of Engineering, National University of Singapore, Singapore, Singapore

Traditional fluorophores including fluorescent dyes/proteins and quantum dots (QDs) are based on 'downconversion fluorescence', emitting low energy fluorescence when excited by high energy light. They have several drawbacks when used in biological applications, for example, photobleaching, autofluorescence, short tissue penetration depth and photo-damage. Upconverting fluorescent nanoparticles emit detectable photons of higher energy in the ultraviolet (UV) / visible (VIS) / near-infrared (NIR) range upon irradiation with NIR light based on a process termed 'upconversion'. They show absolute photostability, negligible autofluorescence, high penetration depth and minimum photodamage to biological tissues. They can be used for ultrasensitive interference-free biodetection/bioimaging because most biomolecules do not have upconversion properties. They are also useful for light based therapy with enhanced efficiency, for example, photodynamic therapy (PDT) of cancer in deep tissues.

IL346

Glyco-functionalised gold nanoparticles for the selective detection of influenza virus and targeted photodynamic cancer therapy

David A. Russell

University of East Anglia, School of Chemistry, Norwich, United Kingdom

Gold nanoparticles are ideal substrates for functionalisation with biological moieties such as carbohydrates and glycoproteins. In this presentation two intrinsic properties of gold nanoparticles, that have been exploited for photobiological applications, will be described. At the nanoscale gold particles exhibit an intense absorption band, due to surface plasmon resonance, the wavelength maximum of which varies with particle size. This property has been used for the selective detection of human influenza virus. The second property, size, has been used to deliver nanoparticles bearing both photosensitiser and a lectin (carbohydrate binding protein) for the targeted destruction of cancer cells using photodynamic therapy. These two properties, together with the ease of particle functionalization, will highlight the photobiological utility of glyco-functionalised gold nanoparticles.

IL347

Powering human civilization in the 21st century: unfolding trends

Nicola Armaroli

Istituto per la Sintesi Organica e la Fotoreattività- Consiglio Nazionale delle Ricerche, Bologna, Italy

During the 20th century, world primary energy consumption has increased over ten times, leading to an unprecedented improvement of the quality of life in some regions of the world. This was made possible thanks to a massive exploitation of fossil fuels that, in the decades to come, has to be significantly moderated due to environmental constraints, particularly anthropogenic climate forcing. Solar energy, in its multifaceted forms, is the most abundant, reliable, sustainable, and safe primary energy source that can profitably replace fossil fuels [1,2]. The transition to a solar-powered world will be a long and difficult process [3] in which some key trends can be envisaged:

- growing share of electricity in energy end use [4];
- increase of efficiency in energy production and consumption [1,2];
- establishment of technologies for the manufacturing of "solar fuels" [5,6];
- recycling of the equipment used for converting renewable energy flows, that is often made of materials available in very limited supply (e.g. precious metals) [7].

The solutions to the tremendous challenge of energy transition require the mobilization of huge human and economic resources in all scientific and technological fields. In this wide context, one selected example will be briefly presented, related to materials for energy-efficient lighting technologies [8].

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IL348

Designing Molecules and Understanding Key Electronic Processes in Organic Photovoltaics

Jerome Cornil

University of Mons, Mons, Belgium

Solar cells based on π-conjugated materials are attracting a growing interest over the last two decades. Progress in the design of materials and understanding of the key electronic processes lead nowadays to power conversion efficiencies around 10%, thus making organic solar cells fully competitive with devices based on amorphous silicon. Moreover, organic-based devices take advantage of the ease of processing, the modularity of chemical synthesis and the possibility to deposit the devices over large areas, even on flexible substrates. The conversion of light into electricity in organic solar cells relies on four successive steps: (i) light absorption and exciton creation; (ii) exciton migration towards a donor/acceptor interface, in analogy to silicon-based cells; (iii) exciton dissociation at the interface; and (iv) charge separation and collection at the electrodes. Each of this step must be optimized to ensure maximal performance for the device. In this talk, I will survey the current state-of-the-art in the understanding of the processes governing the operation of organic solar cells and the challenges to be overcome in the future. I will also illustrate how theoretical modeling can help in accessing a deep understanding at the microscopic level of the key electronic processes and in suggesting new guidelines for the design of materials with enhanced performance.

IL349

Application of photosynthesis to environmental and energy production problems

Massimo Trotta

CNR, Istituto per i Processi Chimico Fisici, Bari, Italy

Some applications of purple photosynthetic bacteria relevant to environmental and energy problems will be discussed:

(1) *Use of photosynthetic bacteria for remediation of environmental sites polluted by heavy metals*. In this application, one of the most stringent parameter, beside microorganism pathogenicity, is the energy source required

for the growth and the metabolism of the microorganisms; photosynthetic microorganisms use solar light, a cheap and largely available energy supplement, which makes them very interesting for many applications. Among photosynthetic microorganisms, purple photosynthetic bacteria represent a small group characterized by the ability of using different energy sources, which allows them to switch metabolism if the environmental condition changes. Purple non-sulfur photosynthetic bacteria possess a large range of metabolic capabilities including anoxygenic photosynthesis, aerobic and anaerobic respiration, and fermentation depending of the growth conditions, and have drawn attention because of their ability to grow under abiotic stress conditions, including the presence of heavy metal ions.[1,2]

(2) *Assembly of organic-biology hybrids in solar energy conversion*. Photosynthetic organisms have been a font of inspiration for the field of artificial photosynthesis[3] since the basic functions of the photosynthetic apparatus were soundly established. The concept of hybrid organic-biologic systems formed by tailored organic fluorophores acting as covalently bound antenna to the RC protein scaffold is being developed in our laboratories. The hybrid functions like the natural system and is capable of outperforming it, under selected conditions, in energy photoconversion[4].

(3) *Organic polymers as scaffolding for photosynthetic enzymes*. Fully functional reaction center from purple bacteria can be reconstituted in synthetic block co-polymer that can self-assemble in water forming called polymerosomes[5], *i.e.* closed vesicles with improved stability than liposomes.

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IL350

Prospects for Algal Bioenergy

Alison Gail Smith

University of Cambridge, Cambridge, United Kingdom

For the potential of microalgae to provide a biofuel feedstock to be realised, it is essential to be able to grow stable and robust cultures at scale. One approach that may contribute to this is to take advantage of the behaviour of microalgae in their natural environment, namely that they exist in consortia with other algae and bacteria, and that this likely results in the exchange of nutrients. This can be illustrated by the fact that over half of all microalgal species require vitamin B12 (cobalamin) for growth. There is no phylogenetic relationship between those that require the vitamin and those that are independent, and instead it appears to be the result of the loss of a single gene, *METE*, which has happened multiple times through evolution. We found that bacteria can supply B12 to algae, and that this results in repression of the *METE* gene – suggesting a mechanism for evolution of vitamin auxotrophy, and also that the natural situation for algae is to live in communities with bacteria.

We have now established a model system between a B12-dependent green alga, and a soil bacterium that provides B12 to the alga in exchange for fixed carbon. We have found that the two organisms establish a remarkably stable co-culture, and appear to be less susceptible to invasion by contaminating bacteria than axenic cultures. Using genomic analyses we aim to establish key molecular features that enable this artificial consortium to operate. Ultimately this will enable the design of other more elaborate algal consortia to facilitate cultivation at scale – a concept we have termed Synthetic Ecology.

OC351

Reconstitution of a fully active photosynthetic reaction center in organic scaffoldings for supramolecular biological-organic hybrid devices

Rocco Roberto Tangorra[1,2], Francesco Milano[1], Alessandra Operamolla[2], John Henrard[3], Roberto Comaparelli[1], Francesca Italiano[1], Omar Hassan Omar[4], Gianluca Maria Farinola[2], Angela Agostiano[2,1], Massimo Trotta[1]

[1]CNR Istituto per i Processi Chimico-Fisici, Bari, Italy; [2]Università degli Studi di Bari Aldo Moro, Bari, Italy; [3]Namur University, Namur, Belgium; [4]CNR Istituto di Chimica dei Composti Organometallici, Bari, Italy

The highly efficient photoconversion ability of the photosynthetic molecular machinery is continuously spurring the assembly of artificial devices, using the photoconverting proteins for technological applications[1]. It is hence of paramount importance to have a robust, durable and versatile scaffold to be loaded with the integer and fully active photoconverter.

In this communication, the photosynthetic reaction center (RC) from the bacterium *R. sphaeroides* is reconstituted in synthetic block co-polymer vesicles where it retains its functional activity. In particular amphiphilic symmetric block co-polymer, based on poly(2-methyloxazoline)-poly(dimethylsiloxane)-poly(2-methyl-oxazoline) (ABA) self-assemble in water[2], forming the polymerosomes, *i.e.* structures resembling phospholipid liposomes but with an inherently higher stability.

The protein loading of polymerosomes was obtained, for the first time, by using the so called micelle-to-vesicle transition (MVT) method[3], a mild, highly biocompatible and well-established technique employed for membrane protein insertion in liposomes. ABA vesicles were characterized by TEM, AFM and DLS techniques. Interestingly, spectroscopic and enzymatic data, directly obtained from the embedded protein, indicate that the RC tends to place in the external portion of the ABA, i.e. the poly(2-methyloxazoline) facing the bulk solution and not, as expected, randomly oriented in the central poly(dimethylsiloxane) core. In these organic vesicles RCs shows, therefore, a much higher activity compared to the analogue vesicles formed by phospholipids[4].

The ABA scaffolding with an asymmetric distribution of RC can eventually be functionalized with opportune organic moieties to form supramolecular assemblies for the design of hybrid bio-organic photoconvertion devices.

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[3] F. Milano et al., *Photosynth. Res.*, 100 (2009), 107-112

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OC352

Development of new dyes for TiO_2 solar cells based on porphyrinic macrocycles

Maria Graça PMS Neves, Ana M. V. M. Pereira, Ana F. R. Cerqueira, Victorina N. Lufilakio, Maria A. F. Faustino, José A. S. Cavaleiro, António F. da Cunha, Mário J. C. Lima

University of Aveiro, Aveiro, Portugal

It is unquestionable that one of the most important challenges of our society is the development of new energy strategies to tackle global warming and exhaustion of fossil fuels. In this context, and inspired by the natural photosynthesis, generation of electrical energy from solar light is a long term research interest, and dye-sensitized solar cells (DSSC) with mesoporous TiO_2 have been regarded as one of the most promising candidates. Porphyrins have been evaluated as photosensitizers for DSSC and have demonstrated performances indistinguishable from those of efficient ruthenium polypyridyl complexes.[1] In continuation of our interest in the conception of models that mimic key steps of the photosynthetic process,[2] we report here the preparation of *β-p*-carboxyaminophenylporphyrinic

derivatives and their efficiency in TiO_2 DSSC devices, using the Ruthenizer 535-bis TBA dye (N719) as reference. The experimental procedure and the spectroscopic data for the new compounds, as well as the fabrication and characterization of DSSC cells, will be shown and discussed.

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IL353

Appropriate Lighting for Maximal Human Health

Joan E. Roberts

Fordham University, Department of Natural Sciences, New York, United States

Lighting may have either a positive or negative effect on human health. The proper spectrum of lighting during the morning and evening leads to a balanced circadian response. As the human immune response is circadian, “in sync” circadian immune response results in vigorous health, while an “out of sync” circadian immune response increases the risk of infectious disease and cancer. Blue visible light (460 – 500 nm) transmitted to the eye is a powerful inducer of the neuroimmune response in the morning while the same wavelengths of light in the evening interfere with a proper neuroimmune response.

The exact spectrum of light used in human studies must be defined. There is a distinct spectral transmission of light through the eye that changes throughout life. UV radiation reaches the retina of children while there is a dramatic decrease in blue visible light (460 – 500 nm) reaching the retinas of the elderly. In order for lighting to have a positive effect it must be both spectral and age specific. Improper lighting directed at the human eye can lead to transient or permanent blindness.

It is now possible to define positive and negative physiological effects of natural and artificial light sources and to devise treatment protocols for circadian disorders. However, in order for these treatments for circadian dysfunction to be reproducible and safe, lighting protocols must use measurements that involve age of recipient, spectrum, intensity, timing and direction.

This symposium will demonstrate the value of proper lighting to human health and well-being as well as the hazards that may occur with improper lighting.

IL354

Circadian Lighting, Sleep and Daytime Functioning

Eus JW Van Someren

Department Sleep & Cognition, Netherlands Institute for Neuroscience and VU Medical Center, Amsterdam, Netherlands

With increasing age, most if not all physiological, behavioral and cognitive 24-hour rhythms become less robust. The biological clock of the brain, located in the hypothalamic suprachiasmatic nucleus and responsible for coordination of all these rhythms, apparently loses some of its orchestrating power. At high age, the clock may need more support from environmental 24-hour rhythms or ‘Zeitgebers’ (Van Someren and Riemersma - Van Der Lek, Sleep Med Rev 2007;11:465-484). Light has been an evolutionary ever-present 24-hr oscillation in the environment that affects sleep-wake regulating systems of the brain.

The first long-term study on light exposure showed that enhancement of the 24-hour rhythm in environmental light improves mood and the sleep-wake rhythm in demented elderly, and ameliorates their cognitive dysfunction (Riemersma-van der Lek et al., JAMA 2008;299:2642-2655). Effects of bright light on mood and the hypothalamus-pituitary-adrenal axis regulating cortisol were confirmed in elderly suffering from major depression (Lieverse et al., Arch Gen Psychiatry 2011;68:61-70). There are strong indications that effects may have been mediated by neuronal plasticity in the hypothalamic suprachiasmatic nucleus (PNAS 2009 106:2490-2494), and that the medial temporal lobe, including hippocampus, is particularly sensitive to sleep-wake rhythm disturbances in elderly people (Nat Neurosci 2009 12:122-123). It is thus worthwhile to implement brighter environments for elderly people, for which practical guidelines can be found at a dedicated website (www.lichtvoorlater.nl).

Not all sleep problems originate in clock malfunction though. We recently initiated the Sleep Registry, an internet survey and task-assessment platform for extensive characterization of different phenotypes of good and poor sleepers (Van Someren et al., Front Neurosci 2009;3:436) (www.sleepregistry.org) with the aim to better understand the brain mechanisms of chronic insomnia and sound sleep.

IL355

Increased Use of Daylight: Promoted by Building Codes and Standards, Supported by Care Providers

Eunice Noell-Waggoner, Robert Dupuy

Center of Design for an Aging Society, Portland, OR, United States

The value and importance of providing daylight in the common living/dining areas of care facilities has at last been recognized by various disciplines. The discoveries of the action spectrum for melatonin suppression and the photoreceptive retinal ganglion cells provided validation that most residents of care facilities are being deprived of circadian light. There is a strong correlation between the circadian aspects of physiology and the common problems of many older people in care facilities, including insomnia, depression and impaired cognition.

Building Codes: Windows are required in resident bedrooms for light and fire exits. The central living/dining areas where resident spend their day were defined as ‘intervening spaces’, similar to corridors, so no windows (daylight) were required.

Best Practices for Design of Care Facilities:

The ‘neighbourhood concept’ arranged bedrooms around the central living/dining spaces to shorten the distance between the two areas, closing out daylight.

Energy Code: In the United States electric energy for lighting was based on the needs of young-middle aged people.

Current Changes:

Codes/Regulations: The Guidelines for Design and Construction of Residential Health, Care, and Support Facilities are accepted as regulations in 43 states. The 2014 Guidelines will require 40% of walls in living/dining areas to be windows.

Best Practices: Care providers have observed that daylight has a positive impact on their residents. They are increasing daylight in their new and existing buildings.

Energy Code: The Energy Code is requiring daylight in all spaces over a certain size. The Energy Code bases electrical energy use on ASHRAE Standard 90.1; the 2013 Standard will increase electric energy for buildings that serve the visually impaired, including care facilities.

IL356

Device for Measuring Melatonin-Suppressive Irradiances of Natural and Artificial Light Sources

Dieter Kockott[1], Helmut Piazena[2]

[1]*Scientific consultant, Hanau, Germany;* [2]*Charite Berlin, Berlin, Germany*

The efficacy of optical radiation for a certain photochemical or photobiological process is described by the associated action spectrum which indicates the relative spectral sensitivity. A well-known example is the action spectrum V(λ) for the visual power of the human eye. The irradiance being effective for the visual process is

$$E_{VIS} = \int E(\lambda)\, V(\lambda)\, d\lambda$$

E(λ) being the spectral irradiance.

From this, the two principal ways result how to measure E_{VIS}.

- Measurement of the spectral irradiance E(λ) with a spectral radiometer followed by a calculation of the spectral irradiance using V(λ) or
- Direct measurement of the spectral irradiance E_{VIS} using an integrating radiometer whose spectral sensitivity s(λ) is equal or sufficiently similar to the action spectrum. This is the principle of the Lux meter. Because of the outstanding importance of the visual process, a new unit was introduced for the irradiance weighted by V(λ) being the illuminance with the unit Lux. This means that Lux-meters inform about the illuminance which is important for the visual process. This information is not relevant for other photobiological processes.

In the years 2001/02, a third sensor in the retina was discovered controlling the melatonin-suppression and other circadian effects. If the action spectrum $s_{mel}(\lambda)$ is known, analogously to the visual process an effective irradiance concerning the melatonin suppression $E_{mel} = \int E(\lambda)\, s_{mel}(\lambda)\, d\lambda$ can be defined and measured directly by using an integrating measuring device. Just this is done by the device MSS 1000 which uses the action spectrum $s_{mel}(\lambda)$ published by Gall and Lapuente, Licht 7/8, May 2002, and described in the German specification DIN V 5031-100:2009 (engl. title: Non-visual effects of ocular light in human beings). MSS 1000 measures directly the melatonin-suppressive irradiance. Technical details including spectral response, cosine error, comparison to spectral radiometer measurements and examples for practical applications will be presented.

OC357

About the interaction of man light colour and space and the lighting design process

Monica Birgitta Säter

Department of Architecture Chalmers University of Technology, Gothenburg, Sweden

Humans always live in an interaction of man, light, colour and space (MLCS). Night and day every second of their lives. When humans go indoors they stay in an interaction of MLCS that is manmade. The interaction that can be seen outdoors is the role model for the design of the indoor interaction. Lighting design is a handicraft that always follows the same basic process of four steps. The goal for the design of the indoor interaction of MLCS is to come as close as possible to the interaction that can be seen outdoors to get the same support psychologically, physiologically and visually as we have when we stay outdoors. Lighting design is today most frequently done by a computer program that uses a static level of light and a light source that do not change in spectral composition. This method is by Säter called computer calculated lighting design process (CCLDP).The use of the handicraft lighting design and the process that show the user an extensive care throughout the whole project named by Säter as the User centered lighting design process (UCLDP) on the contrary design the light in a daylight mimicking way and close to the daylight at the place where the user lives. Here I report that the development of the lighting design method called user centered lighting design in a better way support humans psychologically, physiologically and visually compared to the computer calculated method (CCLP) most frequently used to day.

Keywords: Interaction of man, light, colour and space

Säter, M. (2012). Equilibrium User centered lighting design. Towards the development of a lighting design that is individually psychologically, physiologically and visually supportive. Department of Architecture. Chalmers University of Technology. Gothenburg, Sweden 2012.

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OC358

Melatonin suppression by white light sources and blue light risk

Helmut Franz Piazena[1], Leonora Franke[1], Ralf Uebelhack[1], Stephan Voelker[2]

[1]*Charité- Universitätsmedizin Berlin, Berlin, Germany;* [2]*Technical University of Berlin, Berlin, Germany*

Healthy volunteers of different ages and of free pupil adaptation were exposed by white light of different spectra, irradiances and solid angles of emitted light to proof the applicability of both circadian action spectrum as well as of photobiological basic laws on melatonin suppression. Measured and predicted melatonin suppression caused by different white light spectra showed similar slope and degree in the case of equivalent values of the effective (circadian weighted) irradiance E_c and of both constant geometric conditions and exposure time Δt. This was interpreted as indication of applicability of both the circadian action spectrum as well as of the *van Krefeld* law of additivity on melatonin suppression by broad-band light sources. In contrast, applicability of the *Bunsen-Roscoe* law of proportionality was limited to the ranges $E_{cu} \leq E_c \leq E_{cs}$ and $\Delta t \leq \tau_c$. (E_{cu} and E_{cs} defined the limits between insufficient circadian irradiance (E_{cu}) and circadian irradiance to get saturation of melatonin suppression (E_{cs}). τ_c characterized the time constant of melatonin decomposition which was found of about $\tau_c \approx (60 \pm 15)$ min.) In case of exposure in 2π geometry, E_{cu} ranged at about (0.08 ± 0.03) W m^{-2} for young adults and at about (0.15 ± 0.05) W m^{-2} for seniors. values of E_{cs} were found at about (0.3 ± 0.1) W m^{-2} for young adults and at about (0.6 ± 0.2) W m^{-2} for seniors. In case of light exposures using threshold irradiance E_{cs} but with reduced solid angles (Ω) and adequately increased radiances, melatonin suppression decreased significantly in the range $0.1 \text{ sr} \leq \Omega \leq 0.5 \text{ sr}$ due to significant reduction of the number of exposed receptors on the retina whereas melatonin concentration increased for solid angles below about 0.1 sr. However, increase of radiance is limited by glare. Thus, the criterion $k_{cv} \cdot \Omega > E_{cs}/L_{vG}$ has to be met in order to get sufficient melatonin suppression without glare. ($k_{cv} = E_c/E_v$ defined the relative circadian effectiveness of the light source, L_{vG} the threshold radiance of glare and E_v the illuminance.) A further limitation of light exposures has to be expected due to the risk of photochemical and of photodynamic damage of the retina by blue light components in the spectra of white light sources. Unfortunately, threshold limit values are only available for acute photoretinitis, but still unknown for chronic damages. Therefore, criteria were defined and exemplarily used to evaluate different types of white light sources not only in terms of their relative circadian effectiveness but also by consideration of the relative spectral risks of getting photochemical and photodynamic retinal damages depending on person's age.

OC359

GRP78-targeting subtilase cytotoxin sensitizes cancer cells to photodynamic therapy

Malgorzata Firczuk

Medical University of Warsaw, Warsaw, Poland

Glucose-regulated protein 78 (GRP78) is an endoplasmic reticulum (ER) resident chaperone and a major regulator of the unfolded protein response (UPR). Accumulating evidence indicates that GRP78 is overexpressed in many cancer cell lines, and contributes to the invasion and metastasis in many human tumors. Besides, GRP78 up-regulation is detected in response to different ER-stress inducing anticancer therapies, including photodynamic therapy (PDT). This study demonstrates that GRP78 mRNA and protein levels are elevated in response to PDT in various cancer cell lines. Stable overexpression of GRP78 confers resistance to PDT substantiating its cytoprotective role. Moreover, GRP78-targeting subtilase cytotoxin catalytic subunit fused with epidermal growth factor (EGF-SubA) sensitizes various cancer cells to Photofrin-mediated PDT. The combination treatment is cytotoxic to apoptosis-competent SW-900 lung cancer cells, as well as to Bax-deficient and apoptosis-resistant DU-145 prostate cancer cells. In these cells, PDT and EGF-SubA cytotoxin induce PERK and IRE1 branches of unfolded protein response and also increase the level of CHOP, an ER stress-associated apoptosis-promoting transcription factor. Although some apoptotic events such as disruption of mitochondrial membrane and caspase activation are detected post PDT, there is no phosphatidylserine plasma membrane externalization or DNA fragmentation, suggesting that in DU-145 cells the late apoptosis events are missing. Moreover, in SW-900 cells, EGF-SubA cytotoxin potentiates PDT-mediated cell death, but attenuates PDT-induced apoptosis. In addition, the cell death cannot be reversed by caspase inhibitor z-VAD, confirming that apoptosis is not a major cell death mode triggered by the combination therapy. Moreover, no typical features of necrotic or autophagic cell death are recognized. Instead, an extensive cellular vacuolation of ER origin is observed. Altogether, these findings indicate that PDT and GRP78-targeting cytotoxin treatment can efficiently kill cancer cells independent on their apoptotic competence and triggers an atypical, non-apoptotic cell death.

OC360

Photodynamic therapy with genetically encoded photosensitizers – is it possible?

Marina V. Shirmanova[1], Ekaterina O. Serebrovskaya[2], Sergey A. Lukyanov[2], Ludmila B. Snopova[1], Maria M. Kuznetzova[3], Diana V. Uzhakova[1], Vlad A. Kamensky[4], Irina N. Druzhkova[1], Elena V. Zagaynova[1]

[1]*Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russian Federation;* [2]*Bach Institute of Biochemistry RAS, Moscow, Russian Federation;* [3]*Nizhny Novgorod State University, Nizhny Novgorod, Russian Federation;* [4]*Institute of applied physics RAS, Nizhny Novgorod, Russian Federation*

Currently, in photodynamic therapy (PDT) of tumors there is an urgent need for new photosensitizers and approaches to the treatment. Recent advances in engineering of fluorescent proteins include variants of the proteins with pronounced phototoxic properties that potentially can be considered as genetically encoded photosensitizers for PDT. The phototoxicity of these proteins has been shown previously in the *in vitro* models.

The goal of this work is to evaluate the applicability of the phototoxic proteins KillerRed and miniSOG for PDT of tumors *in vivo*.

The study was performed on subcutaneous HeLa tumor xenografts in nude mice using the cell lines stably expressing KillerRed and miniSOG in different cell compartments. *In vivo* fluorescence imaging was used throughout the selection of the treatment mode to assess photobleaching of the proteins. The tumors were exposed to laser irradiation at two regimens and examined pathomorphologically.

The results of the fluorescence imaging of the HeLa tumors producing KillerRed demonstrate 30% decrease of the intensity for 30 min at the power density 150 mW/cm^2 without heating. Irradiation with higher powers (225 and 300 mW/cm^2) resulted in more effective photobleaching but caused temperature growth (up to 37 and 41°C degrees respectively). Histological analysis of the treated tumors with KillerRed in mitochondria and nuclei and nuclei alone showed significant dystrophic changes in the tissue structure, such as nucleus swelling, cytoplasm vacuolization, cellular and nuclear membrane destruction, and activation of apoptosis.

Tumors expressing miniSOG were hardly distinguished from surrounding tissues on the fluorescence images *in vivo* due to strong skin autofluorescence in blue-and-green spectrum range. Nevertheless, considerable drop of fluorescence was revealed in the extracted specimens of the irradiated tumors. However, no difference between treated and untreated tumors was found either in pathomorphology or growth, which most likely related to the lack of FMN in poorly vascularized HeLa tumors.

Genetic encoding of the photosensitizer in a cancer cell seems very attractive for the promise of highly selective cellular photodamage. The first results in animals show the possibility to induce irreversible cell abnormalities using PDT with phototoxic proteins. However, many questions about the treatment modes, effectiveness of the method and gene delivery have to be addressed.

OC361

Cellular density implication on three human retinoblastoma lines for PDT treatment outcome.

Florent Poyer[1], Carole Danièle Thomas[1], Guillaume Garcia[2], Philippe Maillard[2], Mihaela Lupu[1], Joël Mispelter[1]

[1]*Institut Curie INSERM U759, Orsay, France;* [2]*Institut Curie CNRS UMR 176, Orsay, France*

Introduction: In previous studies, efficacity of our PS, a glycoconjugated porphyrins TPP (p-Deg-O-R-ManOH)3, was shown in vivo, on human retinoblastoma and human colorectal tumor subcutaneous xenografts in nude mice. Moreover, cellular death propagation was observed, called bystander effect. In this study, three lines of retinoblastoma (harvested from three patients and with different genotypes and phenotypes) were treated in order to observe and determine which tissue characteristics are important for triggering the cellular death.

Methods & Results: The same protocol (antivascular and anticellular protocol, two injections with 3 hours-interval and illumination at 650 nm, 10 minutes after the second one) was performed several times. The follow-up was monitored using 1H/23Na magnetic resonance imaging. The sodium ion represents an endogenous probe to evaluate the cellular density. Before the treatment, we put into evidence different cellular density for each studied tumor line. In tumor with low cellular density, the extracellular space is larger with higher total sodium content. The lines with lower densities required two PDT treatments in order to arrest the tumor growth while the denser line required one treatment. Immunohistochemical analysis using cleaved caspase-3 antibody was also performed on harvested tumors at different time after one PDT treatment. The apoptotic propagation was faster for the denser tumor line. It started always from necrotic cells initially generated by PDT.

Conclusions: From a macroscopic point of view, cellular density represents an important factor which should be taken into consideration for a PDT treatment outcome. This cellular density modulates the apoptotic propagation.

OC362

Significant alterations in blood perfusion and oxygen saturation occur during clinical PpIX-PDT

Alison Curnow

University of Exeter Medical School, Truro, United Kingdom

In vivo studies of protoporphyrin (PpIX)-induced photodynamic therapy (PDT) have indicated that substantial oxygen depletion occurs shortly after irradiation commences and the resulting hypoxia has the potential to limit the efficacy of this oxygen-dependent treatment process. Superficial blood perfusion and oxygen saturation have now been monitored clinically and non-invasively during standard dermatological methyl-aminolevulinate (MAL)-PDT, using Laser Doppler Perfusion Imaging (LDPI) and Optical Reflectance Spectroscopy (ORS) respectively, alongside non-invasive PpIX fluorescence monitoring.

Preliminary data indicates that despite a relatively large degree of inter-patient variation in both these measurements of perfusion and oxygen saturation, perfusion significantly increased in actinic keratosis and basal cell carcinomas during irradiation (presumably in an attempt to replenish the oxygen consumed by the photochemical reactions initiated by the light during PDT treatment) with no alterations being observed in Bowen's disease. Significant reductions in localised oxygen saturation were also recorded in all three lesion types at the end of the irradiation period (when compared to pre-irradiation values), with significant effects being observed within the first minute of light irradiation commencing, which opposed the effects observed in perfusion and pain scores, and correlated with PpIX photobleaching.

These studies have demonstrated that it is both possible and feasible to employ these non-invasive technologies to monitor MAL-PDT in real-time whilst routine dermatological treatments are in progress within clinic. In addition, the significant changes in blood perfusion and oxygen saturation observed alongside PpIX fluorescence accumulation/photobleaching have permitted enhanced understanding of the mechanism of action of this treatment modality and with further investigation, may help us to improve and/or extend the clinical application of PpIX-PDT for enhanced patient benefit.

OC363

Tryptophan Modulates the Efficacy of a Potent Photoactivated Platinum Anticancer Complex.

Sukirti Kalra[1], Jennifer S Butler[2], Julie A Woods[1], Nicola J Farrer[2], Peter J Sadler[2]

[1]*University of Dundee, Dundee, United Kingdom;* [2]*University of Warwick, Coventry, United Kingdom*

The octahedral Pt^{IV} complex *trans, trans, trans*-$[Pt(N_3)_2(OH)_2(py)_2]$ is a novel, highly potent phototoxic agent for cancer cells. The high potency of this platinum complex has been linked to the combined effect on cancer cells of the production of azidyl radicals and Pt^{II} photoproducts upon photoactivation with visible (blue) light. The major photoproducts of the complex exert their effect partly through the stalling of RNA polymerase II[1]. We wish to further decipher the mechanism of action of this complex. Photocytotoxicity was studied in A2780 ovarian carcinoma cells and is dramatically decreased by a low concentration (500 μM) of L-tryptophan (Trp)[2]. Platinum uptake is not affected by the presence of Trp in sham-irradiated cells. DNA reactivity of the complex alone and in combination with Trp after irradiation was studied using the comet assay. The cells were treated with increasing concentrations of the Pt^{IV} complex in presence and absence of 500 μM Trp both under irradiated and sham-irradiated conditions. DNA migration from irradiated cells decreased from 60% to 36% (n= 4) with increased complex concentration. This indicates DNA crosslink formation in response to photoactivation of the complex (λ_{max}: 420 nm, 5 Jcm^{-2}). At the highest concentration, maximum crosslinking occurred and the least DNA migration. Conversely, when cells were treated with complex and 500 μM Trp, DNA migration increased from 60% to 83% with increasing concentration of the complex, thus completely antagonising the reduction in migration caused by photoactivating the complex. These changes were significant and confirmed by frequency histograms of DNA migration from each slide. DNA migration remained nearly constant in the absence (≈62%-63%) or presence (≈59%-68%) of Trp in the sham irradiated cells as the complex is inactive in absence of light. Neither blue light nor Trp alone affected the parameters measured. Our findings have potential significance for the clinical use of Pt^{IV} complexes in photochemotherapy. Modulating the photodecomposition of the complex using Trp or related compounds, could ameliorate cutaneous phototoxicity following systemic exposure. Furthermore, the serum Trp concentration has been reported to be decreased in cancer patients *v* healthy controls, thus the effectiveness of the complex could be increased in some individuals.

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OC364

The effect of 5-ALA-mediated Photodynamic Therapy on NUCKS, c-src and C9orf10 (OSSA) induction in vitro.

Piotr Ziolkowski[1], Katarzyna Hotowy[1], Marta Woźniak[1], Kamila Duś[1], Elżbieta Czapińska[1], Beata Osiecka[1], Jacek Wiśniewski[2], Andrzej Gamian[1], Grzegorz Terlecki[1]

[1]*Wroclaw Medical University, Wroclaw, Poland;* [2]*Max Planck Institute, Martinsried, Germany*

There are proteins, which are overexpressed and activated in response to acute oxidative stress, especially tyrosine kinases. They are responsible for many basic cell functions such as transmission of extracellular signals through cellular membrane to cytoplasm or nucleus. Oxidative stress-associated Src activator/ Homo sapiens chromosome 9 open reading frame 10 (Ossa/ C9orf10) protects cancer cells from oxidative stress-induced apoptosis by Src family kinases activation. Recent data indicated also that nuclear ubiquitous casein and cyclin-dependent kinases substrate (NUCKS) may play a role in tumor growth. In present study we examined whether photodynamic therapy with 5-aminolevulinic acid induces NUCKS, c-src and C9orf10 expression in breast cancer cell line, MCF-7.

The concentration of 5-ALA was 6.5 mM. Excitation wavelength was 630+/-20 nm, total light dose 5 or 10 J/cm^2 and irradiance 60 mW/cm^2 Cells were incubated and collected at established time points. Western blot and immunocytochemical studies were performed using antibodies against NUCKS, c-src and C9orf10 (OSSA).

The highest c-src expression was observed at 7 hrs after irradiation and increase of c-src expression was also retained at 24 hrs. Furthermore, at 7 hrs after treatment, we observed very strong OSSA expression and this was also strong at 24 hrs after irradiation as confirmed by WB and immunocytochemistry. Western blot analysis revealed strongest expression of NUCKS at 7 hrs after PDT. At next time points, 18 and 24 hrs, expression of NUCKS decreased and became similar to that of control group. Further studies showed very strong expression of NUCKS following PDT with 5-ALA and light irradiation of 5 J/cm^2. Early, at 0 hr, expression of NUCKS was predominantly seen in nuclei, while at 7 hrs expression of NUCKS was observed in disseminated manner within entire cells in both nuclei and cytoplasm, with prevalence of cytoplasmic staining.

We suggest that NUCKS is involved in cellular responses following PDT, and since parallel induction of NUCKS and proapoptotic marker Bax and inhibition of anti-apoptotic Bcl-2

was observed, this protein might also be involved in induction of apoptosis following PDT. We would also like to emphasize that our results showed high constant expression of OSSA. This high expression of OSSA could protect cancer cells from apoptosis through activation of c-src in response to oxidative stress.

OC365

Interaction of polycationic phthalocyanine photosensitizers with bioluminescent bacterial cells investigated by zeta potential measurements

Marina Strakhovskaya, Valentina Nikitina, Natalia Belenikina, Emma Ivanova, Andrei Rubin

M.V.Lomonosov Moscow State University, Biology Faculty, Biophysics Department, Moscow, Russian Federation

Photodynamic inactivation of bacteria is based on cytotoxic properties of reactive oxygen species (ROS), generated by photoactivated sensitizers. In addition to the well-known difference in sensitivity of Gram-positive and Gram-negative bacteria to photosensitization, the latter also vary depending on strains and species [Strakhovskaya et al., 2009]. The main structure responsible for the overall resistance of gram-negative bacteria to external agents (dyes, detergents, antibiotics) is the outer membrane, the part of the cell wall. Its outer leaflet consists mainly of lypopolysaccharides (LPS) with a high content of negatively charged groups. Uncharged lipophilic as well as anionic hydrophilic dyes are not able to overcome outer membrane permeability barrier and therefore they do not exhibit photodynamic activity towards Gram-negative cells. On the contrary, cationic dyes sensitize both types of bacterial cells. It is considered that electrostatic binding of cationic dyes to the bacterial cell walls is primary stage of photosensitizing process since it makes available sensitive cellular targets for photooxidative destruction. However, there is significant heterogeneity among bacteria species concerning structure of the LPS lipid A and core polysaccharides. Gram-negative bacteria are able to modify LPS, leading to decrease in the density of negative charges on the cellular surface and weakening interaction with cationic compounds. Bivalent cations of calcium and magnesium bound to LPS also influence surface potential. To determine bactericidal activity of photosensitizers in connection with the value of surface potential (zeta potential) of bacterial cells we used bioluminescent genetically engineered strain *Escherichia coli* K-12 TG1 (commercially available biosensor ECOLUM, Russia). The method is based on the correlation between photosensitized bioluminescence quenching and inactivation of bacteria colony forming units [Strakhovskaya et al., 2002]. Incubation of bacterial cells with polycationic metallophthalocyanines ($MPcs^{n+}$) lead to the shift of zeta potential from - (30-35) mV up to its total neutralization or even overcompensation depending on photosensitizer concentration. The ability of $MPcs^{n+}$ to neutralize net negative charge of bacterial cells was reduced in the presence of calcium and magnesium ions. Parallel zeta potential and bioluminescence measurements showed a correlation between the shift of bacteria net negative charge by $MPcs^{n+}$ and their photodynamic effectiveness. The obtained results clearly show that electrostatic interactions contribute essentially to the binding of $MPcs^{n+}$ to bacterial cell surface structures as well as to bactericidal photodynamic activity of these photosensitizers.

OC366

Improvement of photodynamic therapy for cancer with tyrosine kinase inhibitors. Implications for scheduling of anti-angiogenic drugs

Andrea Weiss[1], Judy R van Beijnum[2], Debora Bonvin[1], Hubert van den Bergh[1], Arjan W Griffioen[2], Patrycja Nowak-Sliwinska[1]

[1]Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland; [2]VU Medical Center, Amsterdam, Netherlands

Photodynamic therapy (PDT) is clinically used against certain forms of cancer, however, its use in oncology has been limited. This is due to secondary tissue reactions, resulting in enhanced angiogenesis and accelerated regrowth of treated lesions. Current clinically effective angiogenesis inhibitors may be applied for the improvement of anti-cancer PDT.

We tested four angiogenesis inhibitors and showed synergistic improvement of PDT by axitinib and sorafenib, but not by sunitinib and bevacizumab. Sequencing studies showed that angiostasis applied before PDT did not result in an improved anti-tumor effect. Vascular normalization observed after the angiostatic treatment did not contribute in a better treatment outcome. Nevertheless, PDT followed by angiogenesis inhibition is shown to be an efficient anti-cancer therapy. The presented results can be translated to the clinic for certain tumor types in which PDT has a proven benefit, such as tongue base carcinomas, basal cell carcinoma, or for tumor bed sterilization after mesothelioma surgery.

OC367

ALA-PDT-induced epigenetic changes in mouse cerebral cortex

Anatoly Uzdensky, Svetlana Demyanenko, Svetlana Sharifulina

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

ALA-PDT effects (100 mg ALA/kg, i.p., 4 h; laser irradiation: 633 nm, 15 mW/cm^2, 20 min) on epigenetic processes in the normal nervous tissue, which can be damaged along with a brain tumor, were studied using The Panorama Ab Microarray Gene Regulation Kit (Sigma-Aldrich). The fluorescence study showed ALA-mediated accumulation of protoporphyrin IX in the mouse cerebral cortex at 4 h after i.p. injection of 100 mg/kg ALA (3.1 nM/g tissue *vs* 1.2 in control, $p<0.05$). Histological study demonstrated PDT-induced death of some neurons and alterations of cortical blood vessels. Proteomic study showed more than 30% changes in the expression of some proteins involved in epigenetic regulation of transcription, histone modification, DNA repair, nuclear protein import, and proliferation in the mouse cerebral cortex at 1 or 4 h after ALA-PDT. The suppression of transcriptional activity included dimethylation of histone H3 at lysine 9 and overexpression of histone deacetylases HDAC-1 and HDAC-11. Upregulation of Kaiso, the DNA methylation-dependent transcriptional repressor, also indicated suppression of transcription. Down-regulation of transcription factor FOXC2 and protein PABP, which participates in transcription termination, also showed the negative regulation of gene transcription. Decrease in the level of mitochondrial hABH1, which demethylates DNA and RNA, indicated inhibition of the mitochondrial transcription and translation. Down-regulation of chromatin remodeling factor hBrm/hsnf2a that activates transcription also showed transcriptional suppression. ALA-PDT also down-regulated proteins MTA1/MTA1L1 and PML involved in the double-strand DNA repair. This resulted in overexpression of phosphorylated histone H2AX that initiates the assembly of DNA reparation complex. Overexpression of PRMT5, which methylates arginines in different proteins and regulates signal transduction, RNA processing and proliferation, correlated with overexpression of transcription factor E2F4 and importin α5/7. 5-fold overexpression of transcription factor AP-1/c-Jun could lead to

apoptosis in the mouse cerebral cortex at 4 but not 1 h after ALA-PDT. Thus, the expression of proteins involved in epigenetic regulation and choice of the cell strategy between survival and death has changed after ALA-PDT. Major alterations observed in the first hour after the treatment resulted in suppression of transcription and DNA repair. At 4 h after PDT the expression of proteins involved in regulation of proliferation or death of cortical cells was changed more significantly.

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OC368

Repeated low-dose Pba/PDT treatments stimulate cell growth of prostate cancer cells.

Valentina Rapozzi[1], Emilia Della Pietra[1], Daniela Cesselli[1], Benjamin Bonavida[2], Luigi Emilio Xodo[1]

[1]*Department of Medical and Biological Sciences, Udine, Italy;* [2]*Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, Jonsson Comprehensive Cancer Center, University of California Los Angeles, Los Angeles, United States*

Cell recurrence in cancer photodynamic therapy (PDT) is an important handicap that is poorly understood. It has become clear that nitric oxide (NO) is a modulator of PDT activity. By modifying the NF-κB/YY1/RKIP survival/anti-apoptotic loop, NO can either stimulate or inhibit apoptosis. We have reported that PDT induces the release of NO in prostate cancer cells in a concentration-dependent manner and, hence, modifies the loop function by either inducing or inhibiting tumor cell growth. In the present study, we examined if repeated treatments with a low dose of PDT (40 nM) induce tumor cell growth in prostate cancer cell lines. Experimentally, we used (a) a metastatic (PC3) and a non-metastatic (LNCaP) prostate cancer cells (b) Pheophorbide *a* (Pb*a*), a chlorophyll derivative as a photosensitizer and (c) a white halogen lamp with red filter (660 nm) with a fluence of 0.82 J/cm^2 to irradiate the cells after 3h of Pb*a* incubation. We repeated the treatments 8 times (overall duration: 1 month). Following the last treatment, we determined the cell growth proliferation by FACS analysis and a clonogenic assay. We also measured the protein levels of the NF-κB/YY1/RKIP loop. Since this loop is also linked to the epithelial mesenchymal transition (EMT), we measured E-cadherin and vimentin expression levels. To asses the presence of a more aggressive cell population (comprising cancer stem cells), we treated the cell subpopulation with labeled CD24 and CD44 antibodies and examined their fluorescence values by FACS. Overall, our preliminary findings demonstrated that repeated treatments with a low dose of Pb*a*/PDT in prostate carcinoma cell lines stimulated the growth of a more aggressive tumor cell subpopulation that was resistant to PDT-mediated cytotoxicity.

OC369

p38 MAPK-regulated induction of the autophagic adaptors p62 and NBR1 following PDT promotes autophagic clearance of ubiquitin-aggregates and reduces ROS levels by supporting Nrf2-antioxidant signaling

Julien Verrax[1], Noemi Rubio Romero[1], Michael Dewaele[1], Tom Verfaillie[1], Jacques Piette[2], Patrizia Agostinis[1]

[1]*Laboratory of Cell Death Research & Therapy, Cellular and Molecular Medicine Department, KU Leuven, Leuven, Belgium;* [2]*Virology and Immunology Unit, GIGA, University of Liège, Liège, Belgium*

Emerging evidence indicate that oxidative stress instigates the formation of ubiquitin (Ub)-aggregates substrates of autophagy, through a process requiring the ubiquitin binding adaptors p62/SQSTM1 and NBR1. Here, we have investigated the role of p62 and NBR1 in cell survival following photodynamic therapy-mediated by hypericin (Hyp-PDT), a procedure known to incite robust ROS-based endoplasmic reticulum (ER) stress and autophagy pathways. We found that in normal and cancer cells Hyp-PDT stimulated the formation of p62- and NBR1-associated Ub-aggregates, which co-localized with LC3 and were removed by autophagy, through a mechanism partially regulated by p38MAPK. In line with this, genetic or pharmacological inhibition of p38MAPK reduced p62 and NBR1 levels and aggregates formation, impaired Nrf2 nuclear accumulation/activation and increased photo-oxidative stress and cell death. In p62 deficient cells, or cells lacking p62 along with reduced levels of NBR1 (through siRNA knockdown), aggregates formation was also reduced but these cells displayed attenuated ROS levels, reduced caspase activation and improved survival after Hyp-PDT. The increased resistance to photo-oxidative stress exhibited by cells lacking p62 and/or NBR1 was overruled by the inhibition of the p38MAPK, which restored cytotoxic ROS levels, thus indicating the relevance of this signal in the control of cell viability. Intriguingly, interference with either p38MAPK or p62/NBR1 caused an elevation in the basal level of chaperone-mediated autophagy (CMA), a main cytoprotective pathway in PDT. Taken together these findings evidence that in photosensitized cells a p38MAPK-regulated pathway coordinates p62/NBR1-mediated clearance of cytosolic aggregates and mitigates PDT-induced proteotoxicity. They also reveal that a functional p38MAPK-Nrf2 signal is required to keep ROS levels in check and protect against PDT-induced proteotoxicity, independently on aggregate formation and CMA status.

OC370

The Characterisation of *In Vivo* Protoporphyrin IX Fluorescence and Photobleaching in Non-Melanoma Skin Cancers

Ronan Martin Valentine[1], Catherine Louise Campbell[2], Sally Helen Ibbotson[1], Kenny Wood[2], Tom Brown[2], Harry Moseley[1]

[1]*University of Dundee, Dundee, United Kingdom;* [2]*University of St Andrews, St Andrews, United Kingdom*

Background. Photodynamic therapy (PDT) is an effective but not yet optimised therapy for patients presenting with non-melanoma skin cancers (NMSC) and dysplasia. However, protoporphyrin IX (PpIX) fluorescence may be used to monitor and optimise PDT treatments. PpIX fluorescence is currently used as a diagnostic tool to predict PDT efficacy.

Objective. There is limited *in vivo* PpIX fluorescence data pertaining to NMSC. Here, we recorded PpIX fluorescence signatures at specific time points during PDT to determine PpIX photobleaching decay curves. Also, we ascertained how the PpIX fluorescence signal varied within and between individuals, and from their first to second PDT treatment.

Method. Fluorescence measurements were recorded from 25 patients using an optical biopsy system (OBS), before cream application (control), before treatment, mid-way during treatment and after treatment. One additional measurement was performed on each patient during treatment, where each subgroup (n = 5 patients) had their treatment paused after either 10 seconds, 20 seconds, 30 seconds, 60 seconds or 120 seconds.

Results. Our preliminary results suggest that a single exponential decay curve, which is often the chosen mathematical equation, does not fit our *in vivo* PpIX fluorescence photobleaching data at 635 nm and follows a more complicated dynamic decrease. There is a significant decrease between the PpIX fluorescence observed before treatment and 60 seconds ($p < 0.05$), 120 seconds ($p < 0.05$), mid-way during treatment ($p < 0.05$) and after treatment ($p < 0.05$).

Conclusion. Although fluorescence may be quite intense at the beginning of treatment it quickly decreases during treatment (photobleaching), resulting in a small signal after PDT. Our PpIX fluorescence measurements will be coupled with Monte Carlo (MC) models to further characterise *in vivo* photobleaching in NMSC from both superficial and deeper layers, which should inform and tailor patient-specific clinical PDT.

OC371

Singlet oxygen luminescence enables to monitor oxygen consumption in biological systems consisting of fatty acids

Anita Gollmer, Johannes Regensburger, Tim Maisch, Wolfgang Bäumler

University Hospital Regensburg, Department of Dermatology, Regensburg, Germany

Singlet oxygen ($^1O_2$), the first excited electronic state of molecular oxygen plays an important role in many biological and chemical processes. The cellular processes that involve $^1O_2$ have not yet been fully understood, in particular in photo-induced cell death, e.g. the oxidation of certain proteins and fatty acids. However, generation of $^1O_2$ can immediately change its environment, in particular in biological cells that consist of proteins and lipids which can chemically react with $^1O_2$. We investigated the interaction of $^1O_2$ in a simplified model system consisting of fatty acids and the well-known reference photosensitizer (PS) Perinaphthenone (PN) by direct detection of the luminescence photons of $^1O_2$ at 1270 nm. Such a model system is a first approach to mimic the complex environment of $^1O_2$ in a biological cell which consists mainly of water, sugars, proteins and lipids. In this study, the important issue of oxygen consumption is evaluated which has to be considered during luminescence detection of $^1O_2$. It is known that the luminescence signal of $^1O_2$ is depending on the oxygen concentration of the environment. Cellular components such as lipids represent oxygen consumers due to peroxidation of their unsaturated double bonds. Moreover, the deactivation of the PS triplet T_1 state can provide an indication of the actual oxygen concentration at the site of $^1O_2$ generation as well as the oxygen consumption by biomolecules like fatty acids. The time-resolved $^1O_2$ luminescence signal might become an additional tool for a fast and precise detection of oxygen concentration, even in living cells during PDT.

The aim is to get a better understanding of photosensitized reactions of $^1O_2$ with cellular components such as fatty acids to further improve methodologies, in particular at a cellular level using luminescence spectroscopy.

OC372

Amphiphilic meso(sulfonate ester fluoroaryl) tetrapyrroles as possible theranostic agents: photochemical and biological studies

Agnieszka Laura Adamowicz[1], Janusz M. Dąbrowski[1], Ana V Simoes[2], Mario J.F. Calvete[2], Mariette M. Pereira[2], Luís G Arnaut[2], Grażyna Stochel[1]

[1]*Jagiellonian University, Kraków, Poland;* [2]*University of Coimbra, Coimbra, Portugal*

Porphyrins and their derivatives are molecules widely studied for photodynamic therapy (PDT) purpose, as they naturally absorb light in the range of phototherapeutic window (620 – 850 nm) and have tendency to accumulate in tumors [1,2]. Up to now many tetrapyrrolic photosensitizers (PS) have been successfully applied in some clinical treatments, but while considering their particular characteristics, the need of chemically pure, strongly absorbing light, less toxic and generating reactive oxygen species with high yield still exist [3].

Introducing halogen atom in *ortho* – position of the phenyl ring (in *meso* – position of the tetrapyrrole system) increases the rate of triplet state of molecule formation by causing internal heavy atom effect [3]. What is more, this modification increase the molecule stability in general [3]. Also amphiphilic character of the PSs molecules could be strengthened by introducing hydrophilic groups, eg. sulfonic ones [3]. Also this modification has additional benefit with increased stability of whole system – long side chain could be steric hinder against oxidation [3].

From the other side, molecules combining properties appropriate both for therapy and diagnosis are especially desirable [4]. Such a combination of therapy and diagnostics in the same platform is called theranostics. Additional fluorine atoms in PS molecule give possibility to use it as contrast in magnetic resonance with ^{19}F NMR, or in fluorescence imaging.

In this work set of porphyrins substituted in *meso* – position of the macrocycle by phenyl ring with fluorine atoms and alkyl sulfoester side chains is presented. Firstly, physicochemical properties of these compounds will be presented and influence of number of fluorine atoms in molecule on the following properties will be discussed: absorption and emission properties, octanol/water partition coefficients, triplet states lifetimes, singlet oxygen generation quantum yields, photostability and redox potentials. Secondly, cytotoxicity, cellular uptake and *in vitro* photodynamic effect will be presented. The presented physicochemical properties of these photosensitizers are appropriate for PDT of cancer. We also note that sub-nanomolar quantities of studied porphyrins can be detected in aqueous solution. This can be very useful for diagnosis and adds to the use of fluorinated compounds as probes in magnetic resonance imaging[2].

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OC373

The potential of light-activated indocyanine green to enhance the cytotoxicity of low-dose CDDP chemotherapy in cancer cell lines in vitro

Kamola Kasimova[1], Brian Wilson[1], Ting Luo[2], Qing Bin Lu[2]

[1]*Ontario Cancer Institute/University Health Network, Toronto, ON, Canada;* [2]*University of Waterloo, Waterloo, ON, Canada*

The effect of photodynamic therapy (PDT) combined with *cis*-diamminedichloroplatinum(II) (CDDP) on different cell lines is under investigation. Prior observations have suggested that reactive oxygen species (ROS) generated by a Type II photosensitizer in combination with CDDP can augment the cytotoxicity of the latter. This could improve the therapeutic ratio of CDDP therapy for localized tumors. The mechanism is hypothesized to be based on a dissociated electron-transfer from the excited photosensitizer to CDDP, leading to a more active form of the drug. Using indocyanine green (ICG) as the photosensitizer could enhance the CDDP cytotoxicity throughout a larger tumor volume, due to the ability to excite ICG at the long wavelength of 800 nm.

In this study, the dark toxicity of combined ICG and low-dose CDDP was first tested using cell viability (presto-blue) and clonogenic assays, and increased proportionally with ICG

concentration in the 3 tumor cell lines tested: A549 lung cancer, CT26 CL25 colon cancer, H460 lung cancer. A possible explanation is that ICG influences cellular transmembrane potentials, and so enables a higher CDDP concentration in the nucleus. The enhancement factor depends on the cell type, perhaps due to differences in the apoptotic sensitivity to CDDP. The situation with light-activated ICG was more complex and variable, depending on both the cell line and the response assay. In some instances, there was evidence of an enhanced CDDP efficacy in combination with light-activated ICG. However, this is confounded by the variable efficacy of ICG-PDT itself, which was strongly cell-line dependent. Thus, in the case of A549, the combination of ICG+CDDP+light was more effective than either ICG+light alone or CDDP alone, suggesting a synergistic enhancement of the CDDP cytotoxicity in the presence of light-activated ICG. This would be consistent with the concept of the dissociated electron-transfer mechanism. However, the effect in the other 2 cell lines is less clear and requires further investigation.

PL401

Photochemical internalization (PCI) in cancer therapy: from bench to bedside medicine with a novel drug delivery technology

Kristian Berg[1], Anette Weyergang[1], Colin Hopper[2], Michael Rosenblum[3], Anders Høgset[4], Pål Kristian Selbo[1]

[1]Dept. of Radiation Biology The Norwegian Radium Hospital, Oslo, Norway; [2]UCLH Oral and Maxillofacial/Head and Neck Centre, London, United Kingdom; [3]Department of Experimental Therapeutics, M.D. Anderson Cancer Center, Houston, TX, United States; [4]PCI Biotech, Strandveien 55, Oslo, Norway

The utilization of macromolecules in therapy of cancer and other diseases is becoming increasingly important. Recent advances in molecular biology and biotechnology have made it possible to improve targeting and design of cytotoxic agents, DNA complexes and other macromolecules for clinical applications. To achieve the expected biological effect of these macromolecules in many cases internalisation to the cell cytosol is crucial. At an intracellular level, the most fundamental obstruction for cytosolic delivery of therapeutic macromolecule is the membrane-barrier of the endocytic vesicles. Photochemical internalisation (PCI) is a novel technology for release of endocytosed macromolecules into the cytosol. The technology is based on the use of photosensitizers located in endocytic vesicles that upon activation by light induces rupture of the endocytic vesicles and thereby release of the macromolecules into the cytosol. PCI has been shown to enhance the biological activity of a large variety of macromolecules and other molecules that do not readily penetrate the plasma membrane, including type I ribosome-inactivating proteins (RIPs), gene-encoding plasmids, adenovirus, oligonucleotides and the chemotherapeutic agent bleomycin. In addition, PCI may also enhance the therapeutic effect of targeted macromolecules such as immunotoxins. PCI has been developed for clinical use through design of a novel photosensitizer, disulfonated tetraphenylchlorin (TPCSa, Amphinex®) and selection of an approved drug, bleomycin, that benefits from the PCI technology. The first phase I/II clinical trial has recently been finanlized and a phase II trial initiated. The basic mechanisms, the development towards clinical use and some clinical examples will be presented.

IL402

The Role of the Arylhydrocarbon Receptor (AhR) in UVB-induced signaling responses in human skin cells

Jean Krutmann

IUF –Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

Within the past 2 decades strong efforts have been made to elucidate the molecular basis of the UVB stress response in mammalian cells in general and human skin cells in particular. As DNA is the major chromophore for UVB, it was thought that the UVB response is initiated in the cell's nucleus as a consequence of UVB-induced DNA damage. This concept is supported by numerous studies which show enhanced UVB responses in cells deficient in nucleotide excision repair and diminished UVB responses if irradiated cells were treated with exogenously added DNA repair enzymes. It was however challenged by the observation that UVB stress responses can occur in enucleated cells. Subsequent work established that part of the UVB response is indeed independent of DNA damage and instead involves changes at the level of the cell membrane, such as clustering and internalization of cell membrane-bound growth factor receptors as initiating events. For many years, however, the nature of the responsible chromophore and its localization within the cells remained enigmatic. In 2007 we answered this question by showing that the arylhydrocarbon receptor (AhR) is an integral part of the UVB stress response in human skin cells, and that its activation in human epidermal keratinocytes caused the DNA-damage independent part of the UVB stress response. The AhR is a member of the basic helix-loop-helix protein family and functionally serves as a transcription factor which in its inactivated state is part of an intracytoplasmic complex containing the AhR, a c-src kinase and heat shock protein 90. We showed that the AhR is activated in human epidermal keratinocytes upon exposure to UVB, but not UVA. The chromophore for UVB-induced AhR activation is the amino free acid tryptophan, which is present in the cytoplasm and upon UVB irradiation forms a number of photoproducts including formyl-indolo-3,2-carbazole (FICZ) serving as physiological AhR ligands. We showed that exposure of human keratinocytes to physiologically relevant doses of UVB leads to the intracellular formation of FICZ, the subsequent activation of the AhR signaling pathway and - via two different (a genomic and a non-genomic) signaling pathways – to increased expression of genes. We also showed that the non-genomic pathway elicited upon UVB-induced AhR activation mediates the DNA damage-independent activation of cell membrane associated growth factor receptors and subsequent downstream signaling events of the UVB response. UVB-induced AhR activation is of obvious clinical relevance because it causes increased expression of numerous genes including cytochrome P450 1A1 and 1B1, but also COX-2, MMP-1 and thus most likely contributes to photocarcinogenesis and – aging. In collaboration with an industry partner we have therefore developed a topical AhR antagonist for use in sunscreen products. In recent studies we have discovered that the AhR plays a critical role in the regulation of UVB-induced apoptosis in human epidermal keratinocytes. We have also shown that UVB-induced AhR activation does not exclusively occur in keratinocytes. For example, melanocytes express functionally active AhRs and UVB-induced activation of AhR signaling in melanocytes leads to melanocyte proliferation and / or melanin synthesis.

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IL403

UVA as an oxidative stress: the disruption of redox, iron and heme homeostasis

Rex Tyrrell

University of Bath, Bath, United Kingdom

Historical studies from this laboratory have shown that ultraviolet A (UVA) irradiation of human skin cells generates an oxidative stress and also disturbs heme and iron homeostasis. Part of the evidence is that UVA radiation induces heme oxygenase 1 (which catabolises heme to release free iron) in human skin fibroblasts and melanocytes and directly leads to an immediate increase in the labile iron pool (as a result of both ferritin

degradation and free heme release) in all skin cell types tested. Homeostatic maintenance of cellular redox state as well as iron and heme levels requires exquisite control to avoid potential cell and tissue damage. Using UVA as a model oxidant in skin cells provides a powerful experimental system to understand both the mechanisms for maintenance of cellular redox homeostasis and the regulatory pathways underlying activation of HO-1. Heme oxygenase is not only involved in heme and iron homeostasis but also the traffic of iron through the appropriate compartments to ensure its availability at safe levels for cellular functions. Both oxidative (e.g. UVA) damage to cells and tissue as well as inflammatory responses appear to disturb such homeostatic mechanisms and lead to a rapid up-regulation of heme oxygenase 1 which in turn participates in the restoration of non-damaging levels of heme and iron and prevents further damage. These properties almost certainly underlie the strong antioxidant and anti-inflammatory activity of heme oxygenases.

Changes in heme status and Nrf2 activity are clearly involved in up-regulation of HO-1 by UVA. However, it is the negative regulation of this protein that will be a key factor in maintaining cellular heme and iron levels under stress-free conditions. The role of Bach1 in negative regulation of HO-1 expression following UVA radiation has recently been defined. Under acute UVA stress, Bach1 binds to released heme, loses its DNA binding and is exported from the nucleus allowing transcriptional up-regulation of HO-1. Human keratinocytes are constitutively refractory to HO-1 induction and are UVA radiation resistant). Using knockdown technology we have shown that not only is Bach1 also the dominant factor in persistent negative regulation in these skin cells but that HO-2, which is constitutively expressed in this skin type, plays a key role. Such studies contribute to the understanding of the crucial role played by this stress pathway in maintaining cellular iron and heme homeostasis in UVA damaged human skin as well as in protecting organs and preventing disease.

IL404

Photoprotective and therapeutic effects of the flavonoid Luteolin in human keratinocytes

Marjan Garmyn, Kathleen Barrette, Sofie Van Kelst, Lien Verschooten

University Hospital Leuven, Leuven, Belgium

Flavonoids are botanical compounds which have protective functions in plants and are abundantly present in the historically known 'medicinal plants'. We studied photoprotective and chemotherapeutic potential of Luteolin in normal and malignant, squamous cell carcinoma (SCC)-derived skin cells. We tested the effects of LUT on selected parameters of the sunburn response in normal human keratinocytes (NHK), exposed to physiological doses of UVB. LUT attenuated UVB-induced cell death via delay and inhibition of intrinsic apoptotic signaling. LUT not only predominantly affected the mitochondrial apoptosis pathway through its antioxidant capacity, but changed also the balance of Bcl2-familiy. Furthermore, LUT had inhibitory effects on the UVB-induced release of the inflammatory mediators interleukine-1alfa and prostaglandine-E2. Using different cell lines derived from squamous cell carcinomas (SCC), we demonstrated that LUT did not increase the resistance of malignant keratinocytes to UVB. Furthermore we demonstrated that higher concentrations of Luteolin, which were not toxic for the normal skin cells, could trigger caspase dependent cell death in malignant skin cells. Luteolin-induced apoptosis was accompanied by inhibition of a survival pathway (AKT), and sensitivity decreased with tumor progression, as primary SCC cells (MET1 cells) were considerably more sensitive to Luteolin than the isogenic metastatic MET4 cells. Extensive intracellular vacuolization was observed in Luteolin-treated metastatic (MET4) cells, which were characterized as acidic lysosomal vacuoles, suggesting the involvement of autophagy. Transmission electron microscopy, mRFP-GFP-LC3 assay and p62 protein degradation, confirmed that Luteolin stimulated the autophagic process in the metastatic MET4 cells. Blocking autophagy using chloroquine magnified Luteolin-induced apoptosis in the metastatic SCC cells. Together, these results suggest that Luteolin has the capacity to induce selectively apoptotic cell death both in primary cutaneous SCC cells and in metastatic SCC cells in combination with chloroquine, an inhibitor of autophagosomal degradation. These data underscore the potential of the flavonoid Luteolin as part of new photoprotective strategy and at the same time as possible chemotherapeutic agent.

IL405

UV induced melanogenesis: cellular and molecular aspects

Jo LW Lambert

Department of Dermatology, Ghent University Hospital, Gent, Belgium

Melanogenesis is a complex process that takes place when melanocytes are activated by UVR emitted by the sun, followed by the generation of melanin. In a response, melanocytes protect the skin against the harmful effects of UVR by producing melanin-containing melanosomes, and by transferring them to the neighbouring keratinocytes where the melanin pigment serves as a shield against UV damage. Like most of these processes, distinct signaling pathways, which result in the activation of specific transcription factors, and a whole range of subsequent reprogramming of gene expression, will help the melanocyte to overcome the harmful UV effects. How these processes are delegated and controlled has not yet been completely elucidated. MiRNAs have recently been shown to be regulators of gene expression, and are known to be involved during stress induced responses; but until to date, no miRNAs have been associated with UV stress resulting in the augmentation of ultraviolet-induced pigmentation. Recently we found a link between melanogenesis and miRNA gene regulation, adding a new layer of control during pigmentation. Additionally, this can open new paths towards the discovery and development of innovative therapies for the treatment of patients with skin pigmentation disorders.

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OC406

The role of NADPH oxidase in UVA-induced ROS generation in human skin cells

Jin Zhang, Rex Michael Tyrrell

University of Bath, Bath, United Kingdom

Ultraviolet A (UVA, 320-400nm) induces oxidative stress in skin cells through generation of reactive oxygenase species (ROS). As a source of superoxide anion, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is considered to be a major source of ROS generation after UVA irradiation and is likely involved in various physiological/pathological processes. NADPH oxidase (NOX) is a trans-membrane enzyme and contains NOX1,2,3,4,5 and DUOX1, DUOX2; 7 family members.

We have extended previous work (Valencia and Kochevar, 2008, J. Invest. Dermatol. 128**,** 214-222) on the role of NOX in UVA-induced ROS generation in cultured human skin cells. For this purpose, we examined NOX activity after UVA irradiation in two cultured human skin cell types and observed NOX activation in skin fibroblasts (FEK4) and as well as keratinocytes (HaCaT). In FEK4 cells, ROS generation by UVA irradiation was abolished by the NOX inhibitor Diphenyleneiodonium. Interestingly NOX1 and NOX4 protein levels are up-regulated to different extents after UVA irradiation of the two skin cells types. This may indicate a different roles of the NOX isoforms in UVA induced

skin damage and , accordingly, examination of the role of NOX1 and NOX4 on ROS generation after UVA irradiation in skin fibroblasts using small RNA interference (siRNA) technology provided evidence that the two isoforms influenced UVA-activated ROS generation to quite different extents.
ROS generation following UVA irradiation is much lower in skin fibroblasts with HO-1 gene knock down. The observation that siHO-1 knockdown also decreased NOX activity after UVA irradiation in cultured HaCaT cells is consistent with the conclusion that HO-1 is upstream of NOX.

OC407

UVA-induced optical skin changes

Mantas Grigalavicius[1], Asta Juzeniene[1], Børge Hamre[2], Jakob Stamnes[2], Vladimir Iani[1], Johan Moan[1]

[1]Department of Radiation Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway; [2]Department of Physics and Technology, University of Bergen, Bergen, Norway

UVA-induced optical skin changes at different skin depths were studied using noninvasive reflectance spectroscopy. Reflectance spectra were analyzed using a theoretical cutaneous model with inherent optical parameters. Theoretical model is build on the basis of the radiative transfer simulation tool which simulates radiation transfer through the atmosphere. When suitable volume fractions and distributions of physiological components are chosen, the discrete ordinate radiative transfer code for a coupled air tissue system (C-DISORT) provides results which are in accurate agreement with experimental reflectance spectra. In this way, it is possible to estimate dermal blood concentration and oxygenation, upper and lower epidermal thicknesses, upper and lower epidermal melanosome size and concentrations, and epidermal keratin concentration before and after exposure to UV radiation. Calculations were iterated by using an integrating sphere and a goniometric device as measuring probes.
The UVA-induced (20 J/cm^2, 20 min) pigmentation, so called immediate pigment darkening (IPD) resulted in a significant increase in apparent absorption in the visible region, and slight decrease of absorption in the UV region. Bigger volume fraction of blood is responsible for increased absorption in the visible region, while the redistribution of melanosomes in theoretical model enables to explain increased scattering in the UV region.

OC408

Evidence that lower epidermal layers of human skin have an inherent resistance to UVB-induced cyclobutane pyrimidine dimer formation

Katherine Rankine, Neil K. Gibbs

University of Manchester, Manchester, United Kingdom

UVB (280-315nm) irradiation of human skin induces cyclobutane pyrimidine dimers (CPD) in epidermal nuclei which initiate erythema, photoimmunosuppression, photoageing and skin cancer. When skin is exposed to UVB in vivo, higher CPD levels are observed in nuclei of the upper epidermis with reduced CPD levels in the lower layers. This gradient is traditionally explained by skin optics and the attenuation of UVB as it passes through the epidermis. Using a novel irradiation technique we challenge this explanation and demonstrate an inherent resistance of nuclei in the lower epidermal layers to UVB-induced CPD that is independent of skin optics.
Cryosections (10 μm) of photoprotected buttock skin from healthy subjects (n=3; Skin Type I / II) were mounted on microscope slides and irradiated, perpendicularly from above, with UVB doses emitted from a Phillips TL-12 source. Immediately after irradiation, cryosections were immunostained with TDM2 anti-CPD primary and fluorescent secondary, antibodies. Images were captured using a Keyence BZ-8000 microscope and epidermal CPD staining intensity from 10-70 μm above the dermal-epidermal junction (DEJ) analysed with ImageJ software.
At each dose of UVB (5, 10, 20 mJ/cm^2) there was a linear gradient (R^2= 0.38, 0.67, 0.77 respectively) of CPD staining through the epidermis with CPD levels at 70 μm being approximately 50% greater than at 10΄m above the DEJ. The proportionality of CPD staining to UVB dose was confirmed by linear dose responses at 10 and 70΄m (R^2= 1 and 0.99 respectively).
The epidermal gradient of CPD observed in this study reflects that reported after UVB exposure of human skin in vivo. However, by irradiating skin cryosections perpendicularly, we removed the influence of skin optics and all epidermal nuclei received the same UVB dose. Our data indicates an inherent resistance of basal and supra-basal cell layers to UVB-induced CPD and challenges the dogma that reduced CPD in the lower epidermal cell layers is solely due to optical attenuation of UVB radiation by higher epidermal cell layers.

OC409

Consequences of age and UVB radiation on pro-inflammatory cytokines and matrix metalloproteinases secretion by normal human epidermis keratinocytes.

Isabelle Krolikiewicz-Renimel, Olivier Jeanneton, Robin Kurfurst, Sylvianne Schnebert

LVMH Recherche, Saint-Jean-de-Braye, France

Aging and more particularly skin aging can be divided into two processes: the intrinsic aging which is the natural biological process and the extrinsic aging process due to the effects of our environment and more specifically UV radiation.
UV radiation consequences are activation of the skin aging with epidermis and dermis disturbances. For example, collagen and elastin, dermis macromolecules which are involved in the elasticity and skin structure are altered.
A large part of these modifications is caused by oxidative damage, thus inducing the production of cytokines and some matrix metalloproteinases (MMPs). It is well known that the release of such pro-inflammatory cytokines such as TNF-α, IL-1β or IL-8 can lead to skin chronic micro-inflammation.
The aim of this work was to study the cytokines and MMPs secretion profiles from young and old donors before and after UVB irradiation.
In agreement with the ethical committee, biopsies obtained from Caucasian women abdomen (a group of 25 years old and another of 65 years old) were used to isolate normal human epidermis keratinocytes (NHEK). These cells were cultivated in Epilife medium until confluence. Then, the NHEK were irradiated with UVB 50 mJ/cm^2. After a 24 hours period, the surpernatants were collected and used for the quantification of 39 cytokines and 4 MMPs. The assays were performed based on a multiplexing technology using MagPix device (Millipore, France) and cytokines (HCTO-60K-39) and MMPs (HMMP2-55K-04) assay kits (Millipore).
The results showed that, using cells from young donors, the cytokine secretion profile was modified after UVB irradiation. A high increase of pro-inflammatory cytokines (for example, TNF-α, IL-1β, IL-8) was observed while a moderate stimulation of anti-inflammatory cytokines secretion (for example, IL-10, IL-1 RA, IL-4) was measured.
From the elder donors NHEK, the cytokine secretion profile showed an imbalance between pro- and anti-inflammatory cytokines, with a strong increase for the former and a reduction for the latter. After UVB irradiation, this phenomenon is amplified, reinforcing the skin aging process.
For UVB irradiated on young donors NHEK, MMPs (MMP-2, MMP-4, MMP-9 and MMP-10) secretion profile was similar to that obtained from non irradiated elder donors NHEK. All the MMPs studied were dramatically increased (more than x3), leading to degradation of dermis macromolecules and wrinkle formation.
In conclusion, UV exposures participate in the installation of an inflammatory status by increasing the pro-inflammatory

cytokines and MMPs levels, similar to that observed during the skin aging. Specific cytokines and MMPs could be studied in order to further understand their roles in skin photo-aging.

IL410

Mapping viscosity in cells using molecular rotors

Marina Kuimova

Chemistry Department, Imperial College London, London, United Kingdom

Viscosity is one of the main factors which influence diffusion in condensed media. In a cell viscosity can play a role in several diffusion mediated processes, such as drug delivery, signalling and mass transport. Previously, alterations in viscosity in cells and organs have been linked to malfunction; however, mapping viscosity on a single-cell scale remains a challenge.

We have imaged viscosity first inside lipid mono- and bi-layers and in cells using fluorescent probes, called molecular rotors [1]. In molecular rotors the speed of rotation about a sterically hindered bond is viscosity-dependent [2-3]. This approach enabled us to demonstrate that viscosity distribution in a cell is highly heterogeneous and that the local microviscosity in hydrophobic cell domains can be up to 100 times higher than that of water. These conclusions have been confirmed by monitoring the decay and reaction rates of short-lived excited state of molecular oxygen, singlet oxygen, on a single cell level [3].

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IL411

The role of theory in designing optical biosensors based on unusual photophysical properties

Giuseppe Brancato

Scuola Normale Superiore, Pisa, Italy

In this presentation, I describe possible effective strategies for the rational design and synthesis of optical biosensors tailored at imaging studies of relevant biological targets both in vitro and in vivo. From the theoretical and computational viewpoint, the focus will be on the elucidation of the nature of the most important electronic states for fluorescence imaging and on the structural/environmental effects that affect the most the spectroscopic observables. Here, after a brief introduction of our recently developed ADMP/GLOB [1,2] model, a molecular dynamics method based on an integrated *ab initio*/classical potential using localized basis functions and non-periodic boundary conditions, I present illustrative results about rather unusual photophysical properties of some biocompatible chromophores recently investigated in collaboration with photo-physical laboratories [3,4]. In particular, I will emphasize the role of theoretical approaches to uncover and exploit new photophysical features in applications of synthetic molecular probes.

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OC412

Multispectral FLIM to observe cell metabolism for tumour diagnosis

Angelika Rueck[1], Carmen Hauser[2], Simone Mosch[3], Sviatlana Kalinina[4]

[1]AR, Ulm, Germany; [2]CH, Ulm, Germany; [3]SM, Ulm, Germany; [4]SK, Ulm, Germany

Fluorescence guided diagnosis of tumour tissue is in many cases insufficient, because false positive results are interfering with the outcome. Discrimination between tumour and inflammation could be therefore difficult. Improvement of fluorescence diagnosis through observation of cell metabolism could be the solution, which needs a detailed understanding of the origin of autofluorescence. However, a complex combination of fluorophores give rise to the emission signal. Also in PDD (photodynamic diagnosis) different photosensitizer metabolites contribute to the fluorescence signal. Therefore, the fluorescence decay in many cases does not show a simple monoexponential profile. In those cases a considerable improvement could be achieved when time-resolved and spectral-resolved techniques are simultaneously incorporated [1].

The discussion will focus on the detection of NADH, FAD and 5-ALA induced porphyrins. With respect to NADH and FAD the discrimination between protein bound and free coenzyme was investigated with multispectral FLIM in normal oral keratinocytes and squamous carcinoma cells from different origin. The redox ratio, which can be correlated with the fluorescence lifetimes of NADH and FAD changed depending on the state of the cells [2].

Most of the investigations were done in monolayer cell cultures. However, in order to get information from a more realistic in vivo situation additionally the chorioallantoismembrane (CAM) of fertilized eggs was used where tumour cells or biopsies were allowed to grow. The results of theses measurements will be discussed as well.

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OC413

Recording Transient Fluorescence-Lifetime Effects by Line-Scanning

Vladislav I Shcheslavskiy, Wolfgang Becker

Becker&Hickl GmbH, Berlin, Germany

We present a technique that records transient effects in the fluorescence lifetime of a sample with a spatial resolution along one dimension. The technique is based on building up photon distribution over the distance along the scan, the experimental time after the stimulation of the sample and the arrival times of the photons after the excitation pulses. The maximum resolution at which lifetime changes can be recorded is given by line scan time. With repetitive stimulation and triggered accumulation transient lifetime effects can be resolved with a resolution of about 1 ms.

To demonstrate fluorescence lifetime-transient scanning we used the chlorophyll transients that occur when a plant is exposed to light. The stimulation is the turn-on of the excitation laser. A result for non-photochemical transient shows that the lifetime changes from 560 ps to 310 ps for the period of 13.4 s after the turn-on of the laser. We also present the results on the photochemical transients in chlorophyll which happen on the

time scales from 3 ms to 190 ms. The results on fluorescence lifetime changes in sulfonated aluminum phtalocyanines (AlPcS4) upon excitation with 405 nm and 640 nm picosecond lasers will be presented as well.. Potential applications of Fluorescence Lifetime-Transient Scanning microscopy are experiments in plant physiology, electro-physiology, Ca^{++} imaging of neuronal tissue, and study of fast transient effects in photosensitizers.

IL414

Imaging dielectric constant and local order in living cells by multifunctional fluorophores

Giovanni Signore[1], Gerardo Abbandonato[2], Barbara Storti[2], Antonella Battisti[2], Martin Stoeckl[3], Vinod Subramanian[4], Ranieri Bizzarri[5]

[1]IIT@NEST, Center for Nanotechnology Innovation, Pisa, Italy; [2]NEST, Scuola Normale Superiore and CNR Istituto Nanoscienze, Pisa, Italy; [3]Bioimaging Center (L931) Universität Konstanz, Konstanz, Germany; [4]MIRA Institute, University of Twente, Enschede, Netherlands; [5]CNR Istituto di Biofisica, Pisa, Italy

Fluorescent sensors of polarity and viscosity at nanoscale are particularly interesting for high-resolution microscopy imaging of living cells as these physicochemical properties modulate many cellular processes.[1,2] Ideally, polarity/viscosity probes should fulfill these requirements: a) optical responses (intensity, wavelength-shift, lifetime) predictably related to the environmental polarity or viscosity changes, b) strong brightness for high-sensitivity detection, c) easy conjugation to biomolecules. Conventional probes sense local polarity as expressed by orientation polarizability, which depends in a complicated way on both local static dielectric constant ε and refractive index.[3] Here, we describe for the first time a visible-absorbing/emitting fluorescent probe, structurally similar to the GFP chromophore, which efficiently reports on sole ε with good accuracy both in vitro and in living cells.[4] Notably, we found that Generalized Polarization (GP), a classical parameter for ratiometric imaging in cell microscopy,[1] shows a linear dependence upon the logarithm of ε, thus making the probe an effective indicator of local ε through GP measurements. Our probe is suitable for bioconjugation, and its derivatives can report on local polarity of micelles, LUVs, and protein surfaces in vitro. By confocal microscopy we obtained spatially resolved ε maps for many subcellular compartments, such as endoplasmic reticulum, nuclear envelope, and plasma membrane in cultured CHO cells. From a photophysical point of view, we also demonstrated that this probe behaves as a molecular rotor, allowing for the measurement of environmental fluidity (viscosity) through lifetime. Accordingly, we determined maps of local membrane fluidity in living cells at physiological and non-physiological conditions by Fluorescence Lifetime Imaging (FLIM) in conventional and "phasor" mode.[5]

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IL415

STED-FCS fluorescence microscopy

Christian Eggeling

Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom

Stimulated Emission Depletion (STED) far-field microscopy allows the study of living cells with nanoscale resolution, otherwise impeded by the limited spatial resolution of conventional microscopes. Besides the recording of images, the combination of STED with single-molecule sensitive spectroscopic tools such as Fluorescence Correlation Spectroscopy (FCS) discloses complex dynamical processes hidden to the conventional observations. For example, STED-FCS offers novel insights into important cellular processes, such as lipid-lipid and lipid-protein interactions in the plasma membrane of living cells, and their role in cellular functionality. Improved insights into heterogeneities are realized by recent technological developments of the STED-FCS approach.

IL416

SW 2PE-STED superresolution nanoscopy

Paolo Bianchini, Alberto Diaspro

Nanophysics, Istituto Italiano di Tecnologia, Genoa, Italy

Multiphoton (MP) microscopy has become an essential tool for biological imaging. Combined with transgenic models of disease and genetically encoded or molecular fluorescent probes of cellular function, such as voltage- or calcium-sensitive indicators, MP microscopy is considered the presently best means of studying live brain tissue. Different strategies have been used towards high temporal resolution imaging of three-dimensional specimens. Despite the intrinsic optical sectioning advantage, the spatial resolution of MP microscopy remains diffraction-limited and is suffering from its long excitation wavelengths. By combining MP with super resolution approaches, its spatial resolution can be increased. In addition, a strategy for improving temporal resolution has to be implemented. Stimulated emission depletion (STED) microscopy employing a single wavelength for both two-photon excitation and one-photon depletion has been recently demonstrated. Here we attack the temporal resolution aspect within a two-photon super resolution regime utilizing a resonant scanning approach. The shapes of the resulting PSF and STED "donut", when operating in an enhanced spatial and temporal resolution regime, will be discussed.

OC417

Fluorescent Proteins Photobleaching

Dustin Pearson, Anita Ludwar, Belinda Heyne

University of Calgary, Calgary, AB, Canada

The discovery and cloning of the green fluorescent protein (GFP) represents certainly one of the most important breakthroughs of the 20th century, which has revolutionized our understanding of cellular dynamics. GFP is an unusual protein with the ability to emit visible light upon irradiation. In other words, the protein is acting as a "molecular lantern" illuminating the cellular machinery. Since then numerous colors variants have been discovered or engineered. Albeit unique, these fluorescent proteins (FPs) are not without their faults; for example when excited they are prone to photobleaching. The mechanism leading to permanent photobleaching remains to be elucidated. Recently, FPs have been shown to produce singlet oxygen upon excitation, which in turn has been suggested to be responsible for the protein photobleaching. In order to understand the photobleaching mechanism of FPs, our group started to investigate the interaction between GFP, and its mutants, with singlet oxygen. To our surprise, singlet oxygen induced the formation of a permanent protein dimer, composed of an oxidized fluorescent protein and an intact one. These findings are not without any consequences since fluorescent proteins are often

used as pairs in fluorescence resonance energy transfer (FRET) experiments.

OC418

Developing multivalent sulphonylurea probes for beta cell imaging

Andrej Babic[1], Smaragda Lamprianou[2], Heiner Glombik[3], Hans-Paul Juretschke[3], Paolo Meda[2], Norbert Lange[1]

[1]University of Geneva, School of Pharmacy, Laboratory of Pharmaceutical Technology, Geneva, Switzerland; [2]University of Geneva, Geneva, Switzerland; [3]Sanofi Aventis, Frankfurt, Germany

The development of probes that would specifically bind to the insulin-producing beta cells of pancreas is a prerequisite for the development of an efficient method to non-invasively image the natural history of diabetes, and to evaluate candidate therapeutic approaches. As yet, this development awaits the identification of both a suitable beta cell-specific target, and of a probe that could selectively bind to it. We posited that the simultaneous targeting of a couple of beta cell receptors with multiple copies of partially selective ligands, would improve the cell specificity and binding affinity of a probe, as well as its internalization, all changes expected to be beneficial for beta cell imaging.

To test this hypothesis, we targeted the sulphonylurea receptor SUR-1 with various numbers of glibenclamide molecules, which were modified for conjugation to rhodamine-labelled PAMAM-G5 dendrimers of the 5th generation (Ø ~5.4 nm). The IC50 of the dendrimers carrying 2-4 copies of the same glibenclamide derivatives improved by more than 10 fold, indicating an increased avidity of the multivalent probes. Thus, multivalency favors the binding of a partially selective ligand to insulin-producing cells.

Several probes were synthesized, which differed by either the glibenclamide loading or the modifications of the native sulphonylurea. Those probes featuring good avidity were tested for specificity of the fluorescence labelling they induce in cultures of transformed insulin-producing MIN6 cells, and unrelated cell types (HeLa and Panc-1 cells). Several multivalent probes were found to specifically label MIN6 cells, which express SUR-1, but neither HeLa nor Panc1 cells, which lack this receptor. When the experiments were run at 4ºC, most of the fluorescence signal was at the membrane of MIN6 cells, whereas, when the experiments were run at 37 ºC, a sizable portion of this labelling also appeared within the cytoplasm, indicating a rapid internalization of the probe. Candidate probes were further shown to label primary mouse and human beta cells in the same pattern observed in transformed cell lines. Thus multivalent glibenclamide probes provide for the specific labelling of insulin-producing beta cells.

The study provides the proof of principle that multivalent probes promote the labelling of beta cells by ligands that are not per se ideal when used in isolation. The data open the possibility that multivalent dendrimers may be versatile tools for the non invasive imaging of beta cells, by a variety of methods.

OC419

Bacterial fluorescent proteins as probes for reactive oxygen species in living cells

Karatani Hajime[1], Namikawa Yuki[1], Mori Naomi[1], Yamashita Tomoya[1], Nishikawa Yukihiro[1], Oyama Hiroshi[2], Taketani Shigeru[1]

[1]Kyoto Institute of Technology, Sakyo-ku, Kyoto, Japan; [2]Setsunan University, Neyagawa, Osaka, Japan

Yellow fluorescent protein (Y1-Yellow ; λ_{max} 537 nm) and blue fluorescent protein (Y1-Blue ; λ_{max} 462 nm), originating form a luminous bacterium *Aliivibrio sifiae* Y1, carry redox-active riboflavin 5' phosphate (FMN) and ribityllumazine, respectively. The oxidized forms of Y1-Yellow and Y1-Blue are fluorescent, whereas both are substantially non-fluorescent in their reduced forms. In this study, we have characterized Y1-Yellow and Y1-Blue to apply them for visualization of ROS both in mitochondria and cytosol. As a eukaryote model cell, *Saccharomyces cerevisiae* was mainly used. To visualize mitochondria, a mitochondrial signal peptide was fused with Y1-Yellow or Y1-Blue at the N-terminus site. The genes of interest were cloned into pYES2/CT or pFLAG. In the case of Y1-Yellow, the mitochondrial-signal bound Y1-Yellow emits the inimitable yellow fluorescence only at the mitochondrial site. By inhibiting respiratory electron transport chain in a mitochondrial inner membrane with cyanide, the yellow fluorescence of Y1-Yellow, transported to mitochondrion and processed to liberate the signal peptide, was intensified and at the same time, the mitochondrial cluster formation was evidently observed by the Y1-Yellow fluorescence. This observation may show that the local concentration of ROS was raised by the respiratory inhibition, leading to impairment of mitochondrial membrane. Fluorescence of Y1-Blue expressed in the cytosol was usually weak in post log-phase in contrast to the fact that the fluorescence is bright in late log phase. This does not indicate that the expressed Y1-Blue has been broken down, as judged by its Western blot analysis as a function of time. To such dark cells, the addition of Fe (III) ion, cyanide hydrogen peroxide or staurosuporine made Y1-Blue fluorescence quite strong. This increase in fluorescence may be due to the interaction between the reduced Y1-Blue and ROS derived as a result of the uptake of these substances. The cytosolic Y1-Blue fluorescence was locally quenched around mitochondria when the ROS was transiently ROS generated. This fluorescence quenching may be attributed to the interaction between Y1-Blue and cytochrome c, possibly released from mitochondria damaged by the transiently increased ROS after the uptake of Fe (III) ion, cyanide, etc. From these characterizations, it is concluded that Y1-Yellow and Y1-Blue are promising probes for visualization of ROS and the mitochondrial morphology in living cells.

IL420

UV irradiation of skin alters dendritic cell progenitors in the bone marrow

Prue H Hart, Royce LX Ng, Naomi M Scott, Mary Norval

Telethon Institute for Child Health Research, Subiaco, WA, Australia

The effects of UV irradiation of skin on reducing systemic immune responses are long-lasting (1-3 months). During this time, immune cells in peripheral tissues will be constantly replaced by cells from the bone marrow. We have previously published that $CD11c^+$ cells (dendritic cells, DCs) cultured from bone marrow of mice after UV-irradiation of skin have reduced immunogenicity when transferred into naïve mice, and antigen-specific memory responses are attenuated. Prostaglandin E_2 and an epigenetic process are involved directly or indirectly as the cyclooxygenase inhibitor, indomethacin, and an inhibitor of DNA methylation, 5-Aza-2-deoxycytidine, prevented the development from bone marrow of these less immunogenic DCs. To investigate DC development *in vivo*, bone marrow-ablated mice were engrafted with bone marrow cells from steady-state mice, UV-irradiated mice or mice implanted with pellets releasing PGE_2. Sixteen weeks after bone marrow cell transfer, immune responses dependent on DC function were poor in recipients engrafted with cells from the bone marrow of UVR- or PGE_2-administered mice (UV-chimeric mice, PGE_2-chimeric mice). However, if wild-type bone marrow-derived DCs were injected into the UV- and PGE_2-chimeric mice, immune responses were restored to levels measured in control chimeric mice (i.e. those bone marrow-ablated mice engrafted with steady-state bone marrow cells). This suggested a DC-intrinsic effect. Further, macrophage trafficking into the peritoneal cavity of PGE_2-chimeric mice in response to inflammatory alum was significantly reduced. An effect of prostaglandin E_2 on very early progenitors, possibly haemopoietic stem cells, is suggested.

Further, when pregnant mice were UV-irradiated, DCs differentiating from the bone marrow of progeny were less immunogenic and further suggested an epigenetic effect of UVR. The attenuation of DC immunogenicity by PGE_2 may be important in homeostasis after UVR exposure. These studies suggest that UV irradiation of skin, via PGE_2 production, has long lasting effects on bone marrow dendritic cell and macrophage precursors, such that differentiated cells have reduced immunogenic properties and contribute to UV radiation-induced systemic immunosuppression.

IL421

PDT-mediated damage to lymphatic vessels – mechanisms and outcomes

Jakub Golab[1], Witold W Kilarski[2], Angelika Muchowicz[1], Malgorzata Wachowska[1], Renata Mężyk-Kopeć[3], Patrycja Nowak-Sliwinska[4], Melody A. Swartz[2]

[1]*Medical University of Warsaw, Warsaw, Poland;* [2]*Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland;* [3]*Jagiellonian University, Krakow, Poland;* [4]*CHUV University Hospital, Lausanne, Switzerland*

Photodynamic therapy exerts its anti-tumor effects through direct cytotoxicity towards tumor cells and indirectly through induction of inflammatory response and destruction of blood vessels. While anti-vascular effects of PDT have been intensively explored for the last two decades, relatively little is known about the influence of this treatment on lymphatic vessels. Lymphatic vessels provide an escape route for cancer cells and peritumoral lymphangiogenesis is associated with invasion and dissemination in cancer. Emerging evidence is also implicating lymphatic vessels as modulators of anti-tumor immunity, through alterations in immune cell trafficking to and from the tumor, as well as the inflammatory cytokine environment. Recently, the use of PDT against the peri-tumoral lymphatics was reported. The time is ripe to begin exploring the overall influence of this strategy on host immunity to the tumor. This research can be used to develop new translatable therapeutic strategies as well as build new understanding of how tumor lymphangiogenesis is regulated and affects tumor invasion and immunity.

IL422

UV induced changes in the central nervous system and associated lymphoid tissues is associated with protection from autoimmune disease

Scott Byrne, Felix Marsh-Wakefield, Lai Fong Kok, Sarah Leighton, Gary Halliday

The University of Sydney, Sydney, NSW, Australia

Ultraviolet (UV) radiation from the sun is known to cause cancer, in part due to its ability to induce immune suppression. It has also been found to decrease the risk of developing autoimmune diseases like multiple sclerosis (MS) although the mechanisms involved are not known. To test whether UV activation of immunoregulatory cells is associated with this protection we employed the murine CNS autoimmune model, experimental autoimmune encephalomyelitis (EAE). Exposure to EAE-protecting UV was associated with a significant shift in total lymph node cells away from CNS-draining nodes (lumbar) towards the nodes draining irradiated skin (inguinal). Surprisingly, mice protected from EAE by UV had *elevated* numbers of $CD4^+CD44^{hi}$ activated T cells in skin and CNS-draining lymph nodes. However, this T cell subset was significantly reduced in the CNS itself. At EAE onset and peak disease, UV-induced regulatory B cells ($UV\text{-}B_{Regs}$) were significantly elevated in CNS-draining nodes. Furthermore, the number of $UV\text{-}B_{Regs}$ in lymph nodes significantly correlated with favourable disease outcomes. Very few $UV\text{-}B_{Regs}$ were detected in the CNS itself. Thus, UV protection from autoimmunity is associated with alterations to immune cell trafficking, phenotype and function. This is the first report of a role for UV-induced immune suppression in protection from any autoimmune disease.

OC423

Immune suppression following exposure to UV-radiation is associated with increases in mast cell densities in primary and secondary lymphoid tissues

Lai Fong Kok[1], Gary Halliday[2], Scott Byrne[1]

[1]*Discipline of Infectious Diseases and Immunology, The University of Sydney, Sydney, NSW, Australia;* [2]*Dermatology Research Laboratories, Bosch Institute, The University of Sydney, Sydney, NSW, Australia*

Ultraviolet (UV) radiation causes skin cancer, in part, by suppressing adaptive immune responses. One way UV achieves this is by altering the traffic of mast cells around the body. Exposing C57BL/6 mice to UV 4 times per week for 20 weeks resulted in significant increases in mast cell densities in the skin, lymph nodes and spleen. The density of mature mast cells was also higher in the bone marrow (but not thymus) of UV exposed mice as assessed by flow cytometry. In contrast, the frequency and number of T cells, B cells and DC were unaltered by UV in these primary lymphoid tissues. Mast cell accumulation in peripheral sites such as the skin and lymph nodes is associated with a UV-induced increase in CXCL12. Therefore, to determine whether the increase in bone marrow mast cells was due to changes in CXCL12, we isolated mRNA from bone marrow cells after 6, 12 and 16 weeks of irradiation. However, chronic UV did not significantly increase CXCL12 levels in the bone marrow suggesting another mechanism is involved in UV-induced mast cell increases in the bone marrow. To that end, we have discovered that exposure to chronic doses of UV results in an increase in mast cell progenitors in both the bone marrow as well as peripheral blood. These findings explain not only the increase in mast cells within bone marrow, but also those observed at peripheral sites.

OC424

Protection against UV-induced immunosuppression and carcinogenesis by the flavone luteolin

Christopher T Quinn, Vivienne E Reeve

University of Sydney, Sydney, NSW, Australia

Luteolin (3',4',5,7-tetrahydroxyflavone) is a member of the large group of naturally occurring flavonoid phytochemicals that are of dietary relevance. Luteolin has been identified in many plants including chamomile flowers, celery, green pepper, olive leaves, and peanut hulls, and is recognised for antioxidant and anti-inflammatory properties comparable or superior to the better known flavonoids, apigenin or quercetin, which arise from their polyphenolic structure. Topically applied luteolin has been demonstrated in humans to reduce UVB-induced inflammation, and in cultured cells and mouse skin, to attenuate UVB-upregulation of pro-inflammatory signalling pathways. Luteolin also reduced photocarcinogenesis severity in hairless mice. However luteolin has strong UVA/B absorbance, and the attribution of its topical photoprotective properties to either antioxidant effects or sunscreening effects has not been clarified, nor its potential as one of the more powerfully oestrogenic flavonoid compounds present in the human diet. In this study in which luteolin was applied topically to hairless mice post-irradiation with solar simulating UV radiation (SSUV), we found reduction in the sunburn oedema reaction at 10 and 20 μM, but not 50 μM. Luteolin at 10 – 50 μM protected dose-dependently against the suppression of contact hypersensitivity by SSUV, or by its UVB photoproduct, *cis*-urocanic acid, and reduced the SSUV-induction of the immunosuppressive cytokine IL-10. Furthermore luteolin attenuated the SSUV-inhibition of natural killer (NK) cell activity *in vitro* in a non-linear bell-shaped dose response similar to its anti-inflammatory effect, and reminiscent of the known non-linear dose responses to oestrogen in human

immunity. Since the oestrogen receptor (Er)-binding properties of luteolin are reportedly potent, the abrogation of its photoimmune protection by the anti-oestrogenic drug ICI 182780 was consistent. As hairless mice do not express Er-α in skin, this indicates that luteolin acts immunologically *via* the non-classical Er-β, as in our previous findings with the isoflavonoid equol. Luteolin also appeared to upregulate epidermal Er-β expression after SSUV exposure. As the Er's have antioxidant response elements, this suggests that the antioxidant potential of the flavonoids arises not only from their phenolic groups, but also from pathways induced by Er-β signalling. Finally, luteolin at 25 and 50 μM also reduced photocarcinogenesis in the mice, independently of its UV-absorbance potential, supporting the evidence from others of its potential usefulness in human photoprotection.

PL425

Strategies for targeting cancer stem-like cells with photodynamic therapy

Janet Morgan

Roswell Park Cancer Institute, Buffalo, NY, United States

The existence and identification of cancer stem cells (CSC) as the driving force behind tumor growth and dissemination is controversial, and it is becoming clear that no single model or definition can be used to describe all cancer types. This is partly because all cancers, even those of similar subtypes arise under different conditions. Thus individual variations such as immune competency, origin of tumor cell, genetic phenotype, epigenetic factors and the supporting microenvironment influence the growth and behaviour of the tumor initiating cells. No single marker or combination of markers reliably identifies all CSC within a tumor population, partly because specificity may be limited and partly because they may be absent or expressed at different levels on different clones, or even within clones. Markers may or may not have a functional role in maintaining the stem-like characteristics of a CSC, and removal of a CSC from its environment may affect expression of the marker, or its role in maintaining stemness. Alternatively CSC may evade identification by entering a quiescent state, possibly due to external pressure such as therapy, or hypoxia due to inadequate vascular supply, or another inhibitory influence wherein it does not express an active marker, or awaits a cue to self renew. Increasing evidence suggests that CSC have a degree of plasticity which enables them to flexibly convert between less and more differentiated states depending on internal or external factors. Nevertheless, there are many strategies for targeting CSC, but it may be preferable to target both CSC and more differentiated tumor cells simultaneously to prevent evasive maneouvers. Photodynamic therapy lends itself well to eliminating both CSC and bulk tumor cells, because it can destroy both quiescent and dividing cells, although its intrinsic requirement for oxygen is a mark against it given the CSC often occupy a hypoxic niche. Like all therapies adequate drug needs to reach the target cells for adequate photooxidative cell destruction. Our current method uses drugs which target the hedgehog signaling pathway to induce multiple effects that enhance PDT and tumor response: a) transiently increase vasculature for drug delivery and oxygenation; b) inhibit GLI transcription factor mediated cell proliferation to shrink tumors and differentiate tumor cells; and which simultaneously c) downregulate ABCG2 transcription and translation to minimize efflux of substrate PS and d) to inhibit ABCG2 transporter activity to prevent efflux of of substrate PS for increased PS levels.

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PL426

Engagement of sphingolipids in light-tissue interaction

Mladen Korbelik

British Columbia Cancer Agency, Vancouver, BC, Canada

Sphingolipids (SLs) are one of principal groups of bioactive lipids. Besides their basic function in constituting cellular membranes, SLs have major roles in signalling events regulating key cellular activities including proliferation, differentiation, autophagy and apoptosis, stress responses, motility, angiogenesis, immune responses, and host-pathogen interactions. It is therefore not surprising that SLs are critical participants in many aspects of tissue reaction to light, such as function of retinal photoreceptors, response to ionizing, UV and low-level visible light irradiation. Highlighted will be the relevance of sphingolipids in the response of tumors to photodynamic therapy (PDT). Similar to some other types of cancer therapy like chemotherapy and radiotherapy, PDT has a distinct effect on SL profile owing principally to the induction of de novo ceramide biosynthesis. This de novo ceramide emerges as an important participant in the regulation of autophagy and apoptosis of PDT-treated tumor cells. Ceramide and sphingosine-1-phosphate, two key members of SL family, were recently shown to participate in the process of recognition of PDT-induced damage in cancer cells by immune cells and the ensuing mobilization of these cells for instigation of PDT-elicited inflammatory/immune responses. A variety of agents have been developed as effective SL metabolism-modulating drugs for use as anticancer agents or for other therapeutic interventions. It will be shown that such drugs could serve as adjuvants to PDT in cancer therapy due to their ability to elevate PDT-mediated tumor cure-rates.

PL427

Novel twist in alpha-MSH-mediated protection against UV irradiation of the skin

Markus Böhm

Dept. of Dermatology, University of Münster, Münster, Germany

The melanocortin 1 receptor (MC1R) and its ligand alpha-MSH play an important role in the UV and tanning response of human skin. While the function of MC-1R as a mediator of pigmentation within the epidermis is undisputed with regard to reduction of genotoxic stress and protection against UV-induced skin cancer there is increasing evidence for an increasing number of *direct cytoprotective* actions of the melanocortin system independent of melanin synthesis. Accordingly, alpha-MSH has been found to reduce UVB-induced genotoxic stress in a MC1R and cAMP-dependent manner in epidermal keratinocytes. Moreover, alpha-MSH via the cAMP controls expression of the transcription factor Nrf2 and its downstream phase-II-detoxifying enzymes in both human epidermal melanocytes and keratinocytes. Recent evidence from our laboratory further indicates that alpha-MSH and its receptor protects dermal cells from the impact of UVA and thus photoaging. Accordingly, human dermal fibroblasts (HDFs) pretreated with physiological amounts of alpha-MSH exhibited significantly reduced intracellular levels of hydrogen peroxide after UVA exposure. A functional MC1R was essential for the effect of alpha-MSH since Agouti signalling protein, a natural MC1R antagonist, neutralized the protective effect of alpha-MSH in these cells and HDFs carrying loss of function mutations of *MC1R* failed to respond to alpha-MSH. Importantly, the attenuating effect of alpha-MSH on UVA-induced oxidative stress was paralleled with reduced expression of both MMP1 and MMP3, key enzymes of dermal photoaging. Interestingly, gene silencing of catalase completely abrogated the suppressive effect of alpha-MSH on UVA-mediated accumulation of hydrogen peroxide in HDF. In support of this, alpha-MSH increased enzyme activity of catalase in a time- and dose-dependent manner in these cells. Our findings add a novel twist to our current concept how the cutaneous melanocortin system protects against UV-induced stress in unique and complex manner.

IL428

Multifunctional nanoparticles for PDT treatment and MRI detection of brain tumors

Céline Frochot[1], Régis Vanderesse[2], Thierry Bastogne[3], Olivier Tillement[4], François Lux[4], Muriel Barberi-Heyob[3]

[1]*LRGP, CNRS, Université de Lorraine, Nancy, France;* [2]*LCPM, CNRS, Université de Lorraine, Nancy, France;* [3]*CRAN, CNRS, Université de Lorraine, Nancy, France;* [4]*LPCML, CNRS, Université de Lyon, Lyon, France*

After a brief summary of the nanoparticles developed in the field of PDT [1-3], the presentation will focus on the interest of using of multifunctional nanoparticles for the treatment of malignant gliomas. We previously described the interest to conjugate a chlorin to a peptide targeting neuropilin-1 receptor (NRP-1) over-expressed in tumor angiogenic vessels [4]. To improve our system, we developed gadolinium based multifunctional nanoparticles consisting of a polysiloxane core surrounded by covalently grafted gadolinium chelates for MRI, photosensitizer for PDT and tumor vasculature targeting NRP-1 peptides. Photophysical parameters of the photosensitizers coupled to the nanoparticles have been evaluated, number of photosensitizers coupled optimized [5]. Molecular affinity of the nanoparticles for recombinant NRP-1 protein has been estimated. The nanoparticles conferred photosensitivity to MDA-MB-231 cells [6]. After intravenous injection of the multifunctional nanoparticles into rats bearing intracranial glioblastoma multiforme, we observed a positive contrast enhancement of the tumor tissue by MRI, allowing the optimization of the optical fiber positioning [7]. Optimization of drug-light interval and parameters of excitation are under progress.

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IL429

Photochemical internalization of VEGF121/rGel; a strategy for optimizing tumor-vasculature targeting

Anette Weyergang[1], Lawrence Cheung[2], Michael Rosenblum[2], Khalid Mohamedali[2], Johannes Waltenberger[3], Kristian Berg[1]

[1]*Inst. Cancer Research, Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway;* [2]*Dept. Experimental Therapeutics, MD Anderson Cancer Center, Houston, TX, United States;* [3]*University of Münster, Münster, Germany*

Photochemical internalization (PCI) is a modality for intracellular delivery of drugs which lack an effective transport mechanism into the cell. Such drugs are taken up by means of endocytosis and are transported to the lysosomes where they are degraded before they have exerted their biological effect. PCI utilizes photosensitizers which accumulate in the membranes of these endo/lysosomal compartments. Light exposure causes rupture of these membranes and subsequent cytosolic release of the drug which freely can interact with its target.

PCI of the conventional cytostatic drug Bleomycin has been shown to be highly effective *in vivo* and is currently evaluated in clinical trials with promising results. Bleomycin is, however, probably not optimal for PCI mediated delivery, and therefore cannot fully take use of the technology. Recombinant targeted toxins may, however, be designed to possess all characteristics for an optimal drug to be delivered by PCI. We here present PCI of a recombinant fusion toxin composted of $VEGF_{121}$, and gelonin, a type I ribosome inactivating protein toxin. $VEGF_{121}$/rGel administration has previously been shown to be effective in suppressing tumor xenografts and metastasis. Severe adverse effects may, however, limit the possibilities to obtain complete responses with $VEGF_{121}$/rGel monotherapy. PCI was shown to increase the selectivity of $VEGF_{121}$/rGel in VEGFR2 transfected porcine endothelial cells (PAE) *in vitro*. The PCI treatment also resulted in a dramatic reduction in the dosage of $VEGF_{121}$/rGel to 1/100 as measured by a LD_{90} of 10 nM for the fusion toxin alone compared to 100 pM with the PCI treatment. PCI of $VEGF_{121}$/rGel was *in vivo* administered one week after implantation of CT26.CL25. By day 24 after PCI all animals in the non-treatment control group were sacrificed due to tumor size (>1000 mm^3),while a 50% complete response (CR) was found with PCI of $VEGF_{121}$/rGel. The animals were followed up to day 60 when the experiment was terminated. No tumor regression was observed in CR animals in this time-frame. About 50%CR was also detected in PCI of bleomycin-treated animals. Animals receiving PCI of bleomycin showed an average weight loss of 15% compared to no significant weight loss in the PCI of the $VEGF_{121}$/rGel treated group. This is the first report on PCI of a tumor vascular targeting drug. In conclusion, the present results indicate PCI of $VEGF_{121}$/rGel as a highly selective method for destroying tumor vasculature.

IL430

New developments in functionalized fullerenes for PDT: buckyballs, guns and bullets.

Michael R Hamblin[1], Min Wang[2], Long Y Chiang[2], Rui Yin[1], Felipe F Sperandio[1]

[1]*Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, United States;* [2]*Department of Chemistry, University of Massachusetts Lowell, Lowell, MA, United States*

Fullerenes have been studied for over ten years as photosensitizers that can mediate photodynamic therapy (PDT) applications, yet how any of their particular properties is affected by structural modifications still remains a mystery. It is known that fullerenes are more likely to carry out Type I photochemical mechanisms involving superoxide anion, hydrogen peroxide and hydroxyl radicals than comparably active porphyrinoid photosensitizers. We are studying functionalized fullerenes that have one of more pentacationic arms attached with and without additional tertiary amine arms and appended triphenylamine antennae structures. These molecules are being investigated for antimicrobial PDT of Gram-positive and Gram-negative bacteria and killing of cancer cells. We have found the following interesting structure-function relationships. The addition of an additional tertiary amine arm to the cationic arms already present predisposes towards Type I photochemistry presumably because the tertiary amine nitrogen atoms can “feed” electrons into the fullerene cage as bullets are fed into a machine gun. Excitation with short-wavelength UVA light (360 nm) predisposes towards Type I photochemistry compared to longer wavelength white (400-700 nm) light. The mechanism for this difference is not clear at present. The addition of the triphenylamine antennae shifts the absorption towards the red spectrum as expected but also appears to increase Type 1 photochemistry. Fullerenes with antennae perform better when formulated in micelles, which increases activity and accelerates cell uptake.

IL431

Photodynamic drug delivery in cancer and other diseases

Yabo Wang[1], Thorsten Krueger[1], Elodie Debefve[2], Hubert van den Bergh[2]

[1]*University Hospital (CHUV), Lausanne, Switzerland;* [2]*Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland*

The efficacy of a drug depends among others on the compound of interest (selectively) reaching its target. If injected intravenously factors that may play a role in this include (1) the blood supply to the target area, and (2) the circulation time in the

blood. In many cases the drug must then (3) extravasate from the vasculature, and (4) diffuse via the interstitium to and (5) into the tumor cells. The present talk is about step (3) EXTRAVASATION. Improved extravasation can be attained by several methods, either chemically by using drugs, biologically for instance by inflammation, or physically by changing the temperature or applying radiation. We in clinical tests, and others in preclinical essays, observed enhanced drug extravasation in the case of photodynamic therapy (PDT). PDT damages the cytoskeleton causing endothelial cell rounding and contraction, which in turn leads to the disruption of tight junctions between the endothelial cells. Exposure of the endothelial basement membrane then causes the release of clotting factors including von Willebrand factor and thromboxane which act on the platelets to cause increased platelet-surface and platelet-platelet adhesion, thus locally plugging the vessel. Damage to the endothelium may also influence the Ca^{++} levels and hence the interaction with the surrounding smooth muscle cells inducing vessel contraction. We have also shown that leukocytes may start to adhere to the damaged endothelium ("rolling leukocytes"), which together with the release of histamine can lead to the increase of vascular permeability. Finally, following the damage to membrane lipids others have demonstrated an increase in arachidonic acid which via a series of reactions leads to the increase of thromboxane which also leads to vasoconstriction. These complicated PDT effects thus modulate vasodilating and vasoconstriction agents, as well as disaggregating and aggregating processes. Thus the increase in vessel permeability and INCREASED LEAKAGE can be observed after PDT, as well as (later) blood clotting, vessel constriction and reduced blood flow and finally flow stasis, depending on the applied PDT conditions and vessel diameter. The purpose of this research is to find the optimal PDT parameters/conditions to cause strong locally enhanced leakage of drug(s) which are added to added to the bloodstream prior to or after PDT. We demonstrate the effects of the selective leakage in *in vivo* models including intravital microscopy in the chorioallantoic membrane of the chicken embryo, in the mouse skin fold chamber, and in a rat lung cancer model. Natural tumor selectivity of the observed leakage and drug dosing could be enhanced by the adding of specific chemicals and monoclonal antibodies.

OC432

Photoactivated sunitinib causes endothelial and vascular damage

Patrycja Nowak-Sliwinska[1], Andrea Weiss[1], Judy R van Beijnum[2], Grzegorz Szewczyk[3], Tadeusz Sarna[3], Hubert van den Bergh[1], Arjan W Griffioen[2]

[1]*Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland;* [2]*Angiogenesis Laboratory, Department of Medical Oncology, VU University Medical Center, Amsterdam, Netherlands;* [3]*Department of Biophysics, Jagiellonian University, Krakow, Poland*

Sunitinib is a tyrosine kinase inhibitor that is developed as an angiogenesis inhibitor and is currently used in patients with advanced renal cell carcinoma. The compound inhibits the kinase activity of mainly VEGF and PDGF receptors and prolongs progression free and overall survival of these patients. We have found that sunitinib is sequestered in lysosomes of both tumor cells and endothelial cells. As sunitinib is a fluorescent compound, this can easily be seen using fluorescence microscopy. Interestingly, the exposure of sunitinib cultured cells to light of the wavelength corresponding to the excitation wavelength of sunitinib (λ_{ex} 420 nm), led to immediate photo destruction of the lysosomes. The release of the sequestered compound into the cytoplasm resulted in cell death. We hypothesized that this activity can be used for vaso-occlusion by photodynamic approaches. This was tested in the in vivo model of the chicken embryo chorioallantoic membrane (CAM). We determined the spectral properties of sunitinib and found an absorption peak at 420 nm. Indeed, treatment of the CAM with nanomolar doses of sunitinib and subsequent exposure to 420 nm light resulted in specific angio-occlusion in the treated area. Efficiency was drug- and light dose dependent. We performed this treatment on grafted human ovarian carcinoma tumors on the CAM, as well as on colorectal carcinoma implanted in Balb/c mice. Massive destruction of the vasculature could be observed after the treatment. It is suggested that this regimen can be performed for cancer patients that are treated with sunitinib.

OC433

Dual responsive nanoparticles coupled with phthalocyanine Pc 4 enhance photodynamic therapy by signaling from lysosomes to mitochondria

Anna-Liisa Nieminen[1], Huacheng He[2], Alexander Cattran[1], Peisheng Xu[2]

[1]*Medical University of South Carolina, Charleston, SC, United States;* [2]*University of South Carolina, Columbia, SC, United States*

The major challenge in the treatment of head and neck squamous cell cancer (HNSCC) is how to achieve a high cure rate without compromising the function and cosmetic appearance. Photodynamic therapy (PDT), causing minimal scar and loss of function of treated sites, has been proposed as an alternative for surgery to treat HNSCC. Although efforts have been devoted to the development of nanocarriers for photosensitizers (PS), PDT still has not been widely accepted due the lack of an effective PS delivery system. The goal of this study was to develop a phthalocyanine Pc 4-loaded nanoparticle that selectively targets tumor tissue and is capable of self-expanding to release its payload when triggered by acidic lysosomal pH and elevated intralysosomal redox potential. pH and redox dual responsive nanoparticles (DRN) were prepared by dialysis of a poly[(2-(pyridin-2-yldisulfanyl)-co-[poly(ethylene glycol)]-co-[N-iso-propyl methacrylamide]/Pc 4 mixture after crosslinking initiated by tris(2-carboxyethyl) phosphine. Loading efficiency of Pc 4 was 38.6% and the size of the Pc 4-DRN was 98.6 ± 0.6 nm as determined by dynamic scattering. Pc 4-DRN size increased with increasing redox potential and decreasing pH *in vitro*. To assess sub-cellular localization of Pc 4-DRN, human UMSCC22A HNSCC cells were incubated with Pc 4-DRN (200 nM Pc 4) for 0-20 h. Subsequently, cells were loaded with LysoTracker Green (LTG) and tetramethylrhodamine methylester (TMRM) to image lysosomes and mitochondria, respectively, by confocal microscopy. After 1 h exposure to Pc 4-DRN, Pc 4 fluorescence co-localized with small round spheres representing lysosomes but without co-localization of Pc 4 with mitochondria. At 2 h, the size of many Pc 4-DRN-filled lysosomes had increased, indicating lysosomal swelling. At 20 h, LTG fluorescence became diffuse indicating that most lysosomes had broken down. Also, Pc 4 fluorescence co-localized with mitochondria. After irradiation, Pc 4-DRN induced greater cell killing compared to Pc 4 (100% vs. 35% after 5 h of PDT). Collectively, these data suggest that once Pc 4-DRN are taken up by endosomes/lysosomes, they self-expand and eventually degrade in an acidic environment, resulting in breakage of lysosomal membranes to allow free Pc 4 to be released from lysosomes and transported to mitochondria. The DRN is an effective system to deliver Pc 4 into cells and tissues in a controlled manner.

OC434

On the metabolism of 5 aminolevulinic acid derivatives

Andrej Babic, Nicolas Fotinos, Victorija Herceg, Marino Campo, Norbert Lange

University of Geneva, School of Pharmacy, Laboratory of Pharmaceutical Technology, Geneva, Switzerland

Today, 5-aminolevulinic acid (5-ALA) and its derivatives are commonly used in photomedicine, as porphyrin precursors for the fluorescence detection or the photodynamic treatment of

cancerous and pre-cancerous conditions. Although they gained marketing authorization, and, thus, possess a high degree of security and effectiveness, research in the domain is still active in order to optimize the pharmacokinetics and bioavailability of these compounds. The major challenges, with respect to 5-ALA derivatives developments, are optimized formulations, increased stability, and newly designed derivatives for specific applications. Furthermore, a better understanding of the exact fates of these molecules after their administration has to be explored.

In this context, we synthesized stable derivatives against dimerization, such as 3-amino-3-oxohenadioic-1-methyl-6-methylester (AOMM), in which a carboxymethyl moiety is attached next to the amino group of 5-ALA methylester (MAL). This functionalization was designed to follow the admitted metabolization route of 5-ALA derivatives, and was performed to avoid a dimerization of the substrate, main degradation process occurring in aqueous environment. Therefore, this molecule has to be imperatively activated by an esterase to liberate 5-ALA or its methylester. AOMM was efficiently hydrolyzed, into MAL and 5-ALA, when incubated with porcine liver esterases. Surprisingly, human bladder cancer cells incubated with AOMM were totally unable to produce any type of porphyrins, in contrast to controls incubated with 5-ALA or MAL. Therefore, we have prepared another class of 5-ALA esters that are also stable in an aqueous environment under physiological conditions. Applied to prostate cancer cell lines the drug response curves were similar to those observed for hexylaminolevulinate. Furthermore, some of these compounds formed stable nanomicelles suitable for systemic administration.

OC435

Carbon dots for photodynamic therapy

Petras Juzenas[1], Andrius Kleinauskas[1], Sushant Sahu[2], Pengju George Luo[2], Ya-Ping Sun[2]

[1]Oslo University Hospital, Oslo, Norway; [2]Clemson University, Clemson, SC, United States

Nanotechnology can provide photodynamic therapy (PDT) with promising possibilities. Nanoparticle synthesis procedures are relatively easy giving versatile user-tailored tuning capabilities. Nanomaterials can carry on processes, which organic compounds usually cannot. One such process is nanoparticle-based photocatalysis that can be applied for pollutant decomposition, surface sterilization and cancer therapy.

Carbon being ubiquitous element has attracted attention as a biocompatible material for nanoparticle composition. Most of the work on carbon nanomaterials is so far focused on drug delivery and imaging applications. In this study we present a photocatalytic system based on spherical fluorescent carbon-core nanoparticles.

So-called carbon dots coated with poly(propionylethylenimine-co-ethylenimine) or polyethylene glycol were investigated in human prostate adenocarcinoma cell cultures in vitro. The carbon dots photosensitized the cancer cells when exposed to ultraviolet radiation and blue light. Tuning size of the carbon dots will allow photosensitization with light of longer wavelengths.

Furthermore, carbon dots with a silver shell were investigated as potential photosensitizers in the cancer cells. The cell viability decreased following their exposure to carbon-silver dots along with ultraviolet radiation and blue light. In addition, the carbon-silver dots enhanced the effects of ionizing radiation.

In summary, such carbon dots can be suggested as photosensitizers for photodynamic applications. Carbon-silver core-shell dots can be proposed as a bimodal sensitization platform for biological and medicinal use employing non-ionizing and ionizing radiation.

OC436

Photosensitizer-conjugated quantum dots for photodynamic therapy

Ricardas Rotomskis[1,2], Jurga Valanciunaite[1], Artiom Skripka[1], Simona Steponkiene[1], Gediminas Spogis[1], Gediminas Dauderys[1], Vitalijus Karabanovas[1], Saulius Bagdonas[2], Vilius Poderys[1]

[1]Biomedical Physics Laboratory Vilnius University Institute of Oncology, Vilnius, Lithuania; [2]Biophotonics group of Laser Research Center, Vilnius University, Vilnius, Lithuania

PDT is a treatment process during which the transfer of excitation energy from an excited photosensitizer (PS) to a nearby oxygen molecule occurs resulting in the formation of reactive singlet oxygen ($^1O_2$) or other reactive oxygen species which could initiate cytotoxic reactions in cells and tissues. However, conventional PSs usually have different drawbacks such as, insufficient selectivity for cancer tissue, weak absorption in the spectral range of tissue transparency, low photostability, etc. To address these problems new PSs are being sought together with improved carrier systems, including those based on nanoparticles. Recently it has been suggested that quantum dots (QDs) could be used to improve PDT of cancer as a resonant energy donors for conventional PSs. Their exceptional photophysical properties, especially tuneable emission spectra and high photoluminescence quantum yield, make them extraordinary donors for the Förster resonance energy transfer (FRET) to conventional PSs. Moreover, easy achievable surface functionalization of QDs with biomolecules, such as antibodies, nucleic acids, peptides, etc. can lead to their higher biocompatibility and selectivity to tumour sites. Therefore, the complexation of QDs with PS molecules might help to solve some of the excitation and selectivity problems of conventional PSs, thus greatly enhancing their applicability and efficacy in PDT.

The spectral study on the formation of non-covalent complex between water-soluble, carboxyl-functionalized QDs with lipid coatings and PS chlorin e_6 (Ce_6) in aqueous solution is presented. The excitation of Ce_6 via FRET was studied and generation of $^1O_2$ by such complex was measured. The accumulation and stability of QD-Ce_6 complex in live pancreatic MiaPaCa2 cancer cells were investigated by means of spectral, confocal and fluorescence lifetime imaging microscopy (FLIM). Fluorescence confocal imaging with spectral detection ability showed the uptake of QD-Ce_6 complex in cancer cells: the complex localized in plasma membrane and endocytic vesicles. Fluorescence lifetime imaging revealed Förster resonance energy transfer from QDs to Ce_6 within live cells. Moreover, the phototoxicity of QD-Ce_6 complex under QDs-selective irradiation at 470 nm was evidenced.

The possibility of the QD-Ce_6 complex to be as a candidate for the photosensitized tumour therapy under one and two-photon excitation was evaluated, as well as light-induced damage to cancer cells by the QD-Ce_6 complex was achieved.

OC437

Anticancer activity on glioma cancer cell line (F-98) by using the PCI technique in combination with Bleomycin and Temozolomide

Anette Hauge[1,2], Pål Ellingsen[1,2], Mari Nesbakken[1], Mikael Lindgren[2], Rojlina Manandhar[1], Tora Bardal[3], Anders Høgset[4], Odrun Arna Gederaas[1]

[1]Department of Cancer Research and Molecular Medicine, University of Science and Technology, Trondheim, Norway; [2]Department of Physics, University of Science and Technology, Trondheim, Norway; [3]Department of Biology, University of Science and Technology, Trondheim, Norway; [4]PCI Biotech ASA, Oslo, Norway

In vitro cell studies are very important before clinical trials. Specifically, the photosensitizer meso-tetraphenyl chlorin

disulphonate ($TPCS_{2a}$ - Amphinex®) was used in survival studies of rat glioma cancer cells in combination with the novel photochemical internalization (PCI) technique. The tested anticancer drugs were Bleomycin (BLM) and Temozolomide (TMZ). Glioma cells were incubated with $TPCS_{2a}$ (0.2 μg/ml, 18h, 37 °C) before BLM or TMZ stimulation (4 h) prior to red light illumination (Quanta System, 652 nm, 50 mW/cm^2). The cell survival after BLM (0.5 μM)-PCI (40 s light) quantified using the MTT assay, was reduced about 25% after 24 h relative to controls, and 31% after TMZ-PCI. The supplementing quantification by clonogenic assays, using BLM (0.1 μM), indicated a long–term cytotoxic effect: the surviving fraction of clonogenic cells was reduced to 5% after light exposure (80 s) with PCI, compared to 70% in the case of PDT. In parallel, structural and morphological changes within the cells upon light treatment were examined using fluorescence microscopy techniques. The present study demonstrates that PCI of Bleomycin is an effective method for killing of F-98 glioma cells, but smaller effects was observed using Temozolomide following the "light after" strategy. The results are the basis for further in vivo studies on our rat glioma cancer model using PDT and PCI.

IL438

The immune photodermatoses updated

Rik Roelandts

University Hospital St Rafael, Photodermatology Unit, Leuven, Belgium

There are several arguments that the so called idiopathic photodermatoses have an immunological background. A good example is a polymorphic light eruption. Since long this condition was considered a delayed-type hypersensitivity reaction. The pathogenesis of this condition may be mediated by T-cells and Langerhans cells. Immunosuppression in humans by high level UVB exposure is associated with a depletion of Langerhans cells. In polymorphic light eruption a significant failure of Langerhans cells to migrate from the epidermis as compared to normal skin has been demonstrated. The persistence of UVB irradiated Langerhans cells in the epidermis in polymorphic light eruption might be the result of a lack of TNF-α secretion. Because of the impaired migration, activated Langerhans cells move slowly and accumulate in the dermis where they can stimulate memory T-cells. The reduced expression of IL-4 due to a reduced infiltration of neutrophils favours a TH1 response. There seems to be a dose-response in polymorphic light eruption patients over which the immunosuppression is lacking in comparison to an erythemal response. This may explain why PLE lesions are induced by low UV doses and are rarely seen after severe sunburn.

It has already long ago been suggested that an antigen-antibody reaction is involved in solar urticaria whereby the antigen is produced after solar irradiation. Both a circulated photoallergen and reaginic antibodies have been demonstrated. It is assumed that a photoallergen is formed by irradiation in the skin and in many patients also in the serum or plasma. The photoallergen elicits an immediate type hypersensitivity. Presumably, reaginic antibodies in the serum bind to mast cells, the subsequent solar exposure alters a component in the skin to form the antigen and mast cell degranulation and release of mediators is triggered by an antigen-antibody reaction on the cell surface. The factor responsible for serum transfer is probably IgE. The fact that solar urticaria can be treated by irradiation with longer wavelengths than the provoking ones suggest that photoallergens can be destroyed or converted back into precursors.

For chronic actinic dermatitis there are also several arguments in favour of an immunopathogenesis, such as the immunophenotype of the infiltrate, the therapeutic response to immunomodulatory drugs and the associated contact and photocontact allergies. On the other hand there are also many arguments against a delayed-type hypersensitivity reaction. Contact allergy is less frequent in males than in females and is less frequent in elderly patients as compared to younger ones. In addition, several patients with chronic actinic dermatitis do not have a contact allergy. In several cases the positive tests are not relevant. Avoiding the allergens orphotoallergens does not always give an amelioration of the symptoms. So the final question is if the delayed hypersensitivity reaction is the cause or the result of the chronic actinic dermatitis.

IL439

New aspects of the porphyrias

Gillian Mary Murphy

National Photodermatology Centre, Beaumont and Mater Hospitals, Dublin, Ireland

The porphyrias are a consequence of deranged activity of the enzymes of the Haem biosynthetic pathway. The clinical presentation is widely variable, and patients may present to numerous specialties, therefore because of lack of familiarity of these disorders the diagnosis is frequently missed. This presentation will review the common presentations and newer clinical aspects of porphyria with emphasis on the cutaneous porphyrias and outline the pitfalls in diagnostic techniques. Acquired porphyria may be associated with serious underlying disease therefore awareness of these associations may prove lifesaving for patients. New aspects of porphyria also include insights into the genetic basis of disease and phenotype genotype correlations.

IL440

Xeroderma pigmentosum and related conditions

Robert Sarkany

Photodermatology Unit, St John's Institute of Dermatology, London, London, United Kingdom

Xeroderma pigmentosum is one of a set of rare inherited diseases caused by defects in the repair of damage to DNA.

Hereditary defects in double strand repair cause a set of multisystem diseases many of which are associated with easy sunburn reactions, neurological disease, internal malignancies and premature ageing. These disorders include ataxia telangiectasia, Rothmund-Thomson syndrome, and Werner's dsyndrome.

Hereditary defects in the 'nucleotide excision repair pathway' (which repairs UV damage to DNA) cause Xeroderma pigmentosum, Cockayne's syndrome and Trichothiodystrophy.

It has recently been proven that both trichothiodystrophy and Xeroderma pigmentosum are caused by mutations in the same gene (XP-D).

Xeroderma pigmentosum is particularly important for Dermatologists to be aware of because it is the only DNA repair disorder which causes life-threatening skin disease (skin cancer), because early diagnosis and photoprotection increase life expectancy, and because recent advances hold out the promise of increasing the lifespan and quality:

(5) diagnosis: 40% of patients do not suffer photosensitive reactions. They present with increasing exposed site lentigines , starting around 18 months. Non-photosensitive patients develop more and earlier skin cancers. Early diagnosis is difficult but critical to improve the prognosis.

(6) Prognosis: the first long term follow up study is now complete. Average life expectancy in XP is 32 years. Neurological involvement and metastatic skin cancer contribute to death in equal measure.

(7) Antenatal testing: identification of relevant genes and mutations has enabled antenatal testing to be carried out earlier in pregnancy than was possible previously.

(8) Pathogenesis: the close relationships at the molecular level between trichothiodystrophy, Cockayne's and Xeroderma pigmentosum provide insights into the role of DNA repair in skin cancer, sun burn and neurodegeneration. Improved

understanding of the pathogenesis of neurological disease in XP holds out the possibility of drug trials for XP neurological disease in the near future.

(9) Prophylaxis: potential prophylactic topical therapies to slow the skin disease exist.

(10) Photoprotection: cheap handheld UV meters and practical UV filter films are key to improving prognosis by achieving better photoprotection. Sophisticated psychological approaches are being explored to achieve improved photoprotection.

(11) Multidisciplinary: recognition of the need for centrally organised national multidisciplinary services for patients with rare, complex diseases is leading to new services being set up providing a high standard of care. Such a national service has recently been established in Britain.

IL441

Drug and Chemical Photoallergy – New Drugs with Severe Effects

Margarida Gonçalo

Clinic of Dermatology, University Hospital, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Photoallergy presents mainly as eczema whereas phototoxicity is more heterogeneous (exaggerated sunburn, pseudoporphyria, telangiectasia, hypo/hyperpigmentation or photoonycholysis), but there is an overlap between both. Photoallergy is a T cell mediated reaction but most photoallergens have some inherent phototoxic potential which, as in contact allergy, may induce an innate xeno-inflammation that enhances the sensitization process. Moreover, phototoxic chemicals also induce photoallergy in selected individuals.

At present main exogenous photosensitizers are furocoumarins from plants, UV-filters (benzofenones, octocrylene, cinammates) and drugs, particularly NSAIDs (e.g ketoprofen, piroxicam, celecoxxib), fenotiazines (the anti-histamine promethazine), tetracyclines, fluorquinolones (lomefloxacine, ciprofloxacine) and antifungals (voriconazole). There are interesting relations (cross-reactivity?) between contact allergens/photo-allergens, like the examples of piroxicam and thiomersal/thiosalicylic acid and of ketoprofen, oxybenzone, octocrylene, fenofibrate and the perfume cinnamic alcohol.

Photopatch testing is used for confirming the etiology of photoallergic contact dermatitis, but additional systemic photoprovocation may be recommended in systemic drug photosensitivity. A European photopatch test baseline series, comprising mostly UV-filters and NSAIDs, has recently been recommended by the European Societies of PhotoDermatology and Contact Dermatitis, and the procedures have been standardized.

Apart these acute photosensitive reactions that usually clear on drug suspension, there is an increasing concern on the possibility that these drugs induce auto-immunity (lupus erythematosus induced by thiazides, terbinafine, proton pump inhibitors) and enhance photo-immunossupression and photocarcinogenesis. Long term use of photosensitive drugs is associated with an increased risk of actinic keratosis, squamous cell carcinomas and, more worrying, possibly also melanomas, as reported for voriconazole. Moreover drugs recently introduced in the market like imatibinib, vendatinib and, particularly, verumafenib, the BRAFmutation inhibitor used in the treatment of metastatic melanoma, cause severe photosensitivity that may limit its use. Moreover we may question if the increase in squamous cells carcinomas and melanomas with this class of drugs also is related to photosensitivity and enhanced photocarcinogenesis.

IL442

Ultraviolet Exposure at Home and Work in Patients with Photodermatoses

Harry Moseley

The Photobiology Unit, University of Dundee, Ninewells Hospital & Medical School, Dundee, United Kingdom

Patients with photodermatoses have learned through painful experience of the risks associated with exposure to sunlight, and have generally developed appropriate protective strategies. Artificial lighting has been fairly stable for many years. Incandescent lamps do not emit significant levels of UV, and fluorescent lamps are positioned at some distance from the individual. This has now changed as a result of new EU legislation requiring the phasing out of incandescent lamps to be replaced by new energy-efficient lighting. This has raised concerns among photodermatoses patients who are anxious about the possible risks from lamps with significant UV levels being in close proximity to the skin.

We have carried out an extensive investigation of the UV emissions from a total of 182 energy-efficient lamps, comprising compact fluorescent lamps (CFLs), halogen lamps and light emitting diodes (LEDs). Two types of CFLs were identified and these were designated single envelope and double envelope. Mean UVC and UVB emission at 5 cm from single envelope CFLs was 2 mW/m^2 and 105 mW/m^2, respectively. With double envelope CFLS, the respective mean values were 0.03 mW/m^2 and 4 mW/m^2. In the case of LEDs, there was no detectable UVC or UVB (<0.01 mW/m^2).

Photodermatoses patients were tested to a single envelope CFL lamp at a distance of 5 cm. Patients with chronic actinic dermatitis were the most sensitive, with 16 out of 28 testing positive. In all, 29 out of 134 actively photosensitive patients tested positive. 10 patients, who had positive responses to the single envelope CFL, were further tested with the double envelope CFL. In 6 of these patients no erythema was induced; the other 4 patients had responses that were reduced in erythemal grading. For all UV sensitive patients the LED responses were completely negative.

An 81-year-old man with known CAD was referred for further investigation. Despite strict sun protection, to the extent of daylight avoidance, his skin failed to settle. The patient had also noted redness of exposed sites following proximity to light emitting diodes (LEDs) recently installed at home. Monochromator phototesting revealed severe photosensitivity throughout the UV and visible radiation wavelengths (305 nm to 430 nm). Phototesting to a single envelope CFL produced erythematous oedematous responses at 7 hours, which subsequently became purpuric at 24 hours. CFL testing through UV-absorbing Dermagard film produced a less marked oedematous response. LED phototesting resulted in erythema without oedema at test sites. This case highlights profound photosensitivity in CAD complicated by abnormal reactions to both CFL and LED lighting. This patient was sensitive not only to UV but to visible (violet/ blue) light. Most current LEDs for indoor lighting produce higher levels of short wavelength visible light than traditional incandescent lamps. This explains why the patient also had a problem with LED lighting.

CFLs have the potential to aggravate the skin of photosensitive individuals when situated in close proximity. Double envelope CFLs reduce the risk of skin aggravation. LEDs offer a safer alternative for individuals with UV skin photosensitivity but they may not be suitable for individuals whose sensitivity extends into the short visible wavelengths.

Acknowledgement: This work was performed as part of a Masters thesis by Ms Leona Fenton (Johnson).

IL443

Photophysical properties and formulation of curcuminoids

Hanne Hjorth Tønnesen

University of Oslo, Oslo, Norway

The naturally occurring compound curcumin has a potential as a photosensitizer in photodynamic therapy (PDT). Upon excitation curcumin becomes phototoxic to bacteria and mammalian cells via mechanisms that are still to be elucidated The photochemical properties of curcumin are however, strongly influenced by the environment. Further, the compound is nearly insoluble in aqueous media at pH<7, it undergoes a rapid hydrolysis at pH>7 and it is photolabile. The aqueous solubility and hydrolytic stability are increased in the presence of alcoholic cosolvents, micelles or cyclodextrins while the photochemical stability is reduced. Determination of the S_1 dynamics and identification of the deactivation pathways of curcumin and other biologically active curcuminoids may be relevant in assessing the molecular mechanisms that lead to the photosensitizing activity. Assessment of the dependence of the S_1-decay mechanisms on both the molecular substituents and the environmental conditions is a relevant step towards the rational design of synthetic curcumin analogues featuring enhanced biological activity and improved stability. Any decay mechanism competing with that triggering the photosensitizing reactions should by inhibited as much as possible. In the present work a series of curcumin analogues were synthesized and characterized with respect to the influence of molecular substituents and reaction medium on the S_1-decay mechanisms and *in vitro* phototoxicity in bacteria and cells. Topical preparations (e.g., solid foam, lyophilized powder) containing curcumin and selected excipients intended for application in aPDT of wounds and in the oral cavity have been developed

OC444

Degradation of ibuprofen and paracetamol drugs in ultrapure and river water with sunlight and a singlet oxygen photosensitizing material

David García-Fresnadillo[1], Antonio José Sánchez-Arroyo[1], Eduardo Díez-Mato[2], Fernando Carlos Cortezón-Tamarit[1], María Dolores Marazuela-Lamata[2]

[1]Dpt. Organic Chemistry, Faculty of Chemistry, Complutense University, Madrid, Spain; [2]Dpt. Analytical Chemistry, Faculty of Chemistry, Complutense University, Madrid, Spain

The introduction of stringent water quality standards continues to provide significant challenges for a sustainable water future. Therefore, the presence of micropollutants in surface waters has become a problem of environmental and health concern. The combination of photosensitized production of singlet oxygen ($^1O_2$) and solar energy is gaining increasing interest as an effective treatment for disinfection and/or elimination of organic pollutants in wastewater. This work reports a study of $^1O_2$ mediated photodegradation of two commonly used drugs, ibuprofen and paracetamol, that are currently considered as water micropollutants. Photodegradation of the contaminants can be carried out using a $^1O_2$ photosensitizing material and sunlight. The influence of variables such as the radiation dose, hydrophobicity and ionization state of the micropollutant, and water composition is discussed.

Production of $^1O_2$ is achieved by the use of a photosensitizing material consisting of tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) chloride immobilized in a porous poly (dimethylsiloxane) inert support. The $^1O_2$ photosensitizing material, which can be used in compound parabolic collector solar reactors, has been photophysically characterized.

Micropollutant photodegradation results in ultra-pure water show that ibuprofen is susceptible of efficient photodegradation by $^1O_2$, while paracetamol shows reluctance to react with $^1O_2$. The matrix effect observed in river water samples can be attributed to the presence of dissolved organic matter and other inorganic ions (e.g bicarbonate, sulphate, etc) that compete with the organic substrates for the $^1O_2$ and for the cationic sites on the surface of the photosensitizing material, respectively.

OC445

Photaddition of a ruthenium complex(II) on tryptophan. Determination of the photoadducts structure

Virginie Rahal, Jean Christophe Garrigues, Jean Pierre Souchard, Patricia Vicendo

Laboratoire des IMRCP, UMR 5623, Toulouse, France

Due to their photoredox properties, ruthenium (II) complexes can react with biological targets by different photochemical mechanisms (type I, type II). Depending on their ligands, it will be possible to modulate their photo-reactivity. Thus when the target is DNA, these compounds may find numerous applications such as photonucleases, alkylating agents of DNA. In previous work, we demonstrated that some of these compounds that are highly photo-oxidant, could also react with proteins such as the superoxide dismutase Cu / Zn via a complex mechanism involving two successive electron transfer processes. This original reaction would involve an amino acid with a low redox potential, located on the surface of the protein, such as tryptophan. To confirm this hypothesis we studied the photosensitisation of tryptophan, tyrosine and of a tripeptide glu-trp-glu by ruthenium (II) complexes. By EPR studies and flash photolysis investigations, we have shown the formation of the transient species Ru^{1+} and the tryptophan radical cation upon irradiation of trisbipyrazine ruthenium (II) complex ($Ru(bpz)_3^{2+}$), in the presence of tryptophan and tripeptide. $Ru(bpz)_3^{2+}$, which is a strong photo-oxydizer, may at the excited state oxidize tryptophan via an electron transfer process. The photoproducts generated during this reaction were analyzed by mass spectrometry. This study revealed the formation of photoadducts between the tryptophan and $Ru(bpz)_3^{2+}$. The structures of these photoadducts were clearly identified and will be presented. This result tends to prove that the formation of photoadducts [ruthenium complex-tryptophan] on the SOD might be partly responsible of the enzyme inhibition. This study will have interesting applications in the development of new photo-activable agents for photodynamic therapy.

IL446

Photochemistry and photochemical degradation of pharmaceuticals in aqueous solutions under UV-C and V-UV irradiation

Esther Oliveros, Marion Martignac, Marie-Thérèse Maurette, Florence Benoit-Marquié

Laboratoire des IMRCP, UMR/CNRS 5623, Université Paul Sabatier, Toulouse, France

Pharmaceuticals were first detected in the aquatic environment in the 1970s [1]. Since the 1990s, due to improvements in analytical instrumentation, several pharmaceutical compounds have been found in surface waters (sea, rivers…), ground waters and drinking water at low concentrations (ng/L) [2,3]. This contamination is largely of human and veterinary origin (excretion of the drugs either unaltered as the parent compounds or as metabolites) [4], but may also originate from manufacturing wastes. Sewage Treatment Plants (STP) are currently not designed for the removal of these compounds at trace levels. The reported concentrations are much lower than those applied for therapeutic use. Nevertheless the related potential effects on human health associated with chronic exposure, albeit at low levels, are still poorly known [5].

In the last decades, Advanced Oxidation Processes (AOPs) have improved the efficiency of oxidative degradation of toxic and non-biodegradable organic compounds, including pharmaceuticals. Among AOPs, photochemical technologies are mostly based on the production of the hydroxyl radical (HO•), a

powerful oxidizing species, that may achieve complete mineralization of organic pollutants in aqueous solutions [6].
We have investigated the degradation of various types of pharmaceuticals (analgesics, anticancer drugs, antibiotics…) by AOPs using UV-C photolysis of H_2O_2 and/or V-UV photolysis of water for the production of HO• radicals. Efficiencies of the treatments employed have been compared at laboratory and pilot scales, by following the kinetics of the removal of the selected pharmaceuticals by LC-MS/MS. For most compounds, the detection limit was reached within a few minutes. The implications of the UV-C photolysis of these drugs have also been evaluated. The results show that more toxic products may be formed and raise the question of the use of low pressure Hg lamps (germicidal light sources emitting at 254 nm) for the disinfection of drinking water containing organic aromatic residues that absorb at this wavelength.

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IL447

Photostability of illicit drugs in different matrices

Giorgia Miolo

Department of Pharmaceutical and Pharmacological Sciences, School of Medicine, University of Padova, Padova, Italy

An important class of new compounds to be monitored from the photobiological point of view are the illicit drugs (i.e., cocaine, opiates, amphetamines, cannabinoids, methadone) which are present, along with many other organic compounds such as pharmaceutical products, cosmetics and pesticides, in the urban wastewaters, mainly in domestic effluents and hospital services. These substances and other drugs, recently introduced into the illegal market, are part of the new environmental pollutants. The common purification systems (wastewater treatment plants, WWTPs) are not in fact able to eliminate them; indeed, sunlight and certain photocatalytic treatments may increase their toxicity, specially when photooxidation products are formed. Although the concentrations are very low, the risk to humans is linked to a continuous long-term exposure through the drinking water and the food chain. The lack of information about the fate and toxicity of these compounds and/or their metabolites in the aquatic environment, particularly under the effect of sunlight, makes necessary the study of their photodegradation processes.
As regards the study of illicit drugs photostability, another interesting aspect concerns their presence in the keratinic matrix, especially in the hair samples, where they are analysed along with their metabolites in toxicological investigations to quantify drug consumption or exposure. The action of sunlight can take place directly on the molecules of the drug or can be mediated by radicals and reactive oxygen species produced by eumelanin and pheomelanin, present in the hair, under the effect of irradiation. Therefore, variations in the concentration of the abuse substances may distort the toxicological investigations giving incorrect drug levels or even false negative results. On the other hand, the formation of new photodegradation products from illicit drugs could lead to the identification of new useful markers of abuse in forensic medicine. Some examples of illicit drugs (i.e. cocaine, delta-9-tetrahydrocannabinol, morphine and EDDP, the main inactive metabolite of methadone) photodegradation under UVA-UVB light both in hair samples and in aqueous solution will be presented.

IL448

Photodehalogenation of drugs: chemical mechanisms and biological implications

Virginie Lhiaubet, Miguel Angel Miranda

Instituto de Tecnologia Quimica, Valencia, Spain

Photosensitizing effects of xenobiotics is currently a matter of public health concern, since modern lifestyle often combines sunlight exposure with the presence of chemical substances in the skin. Indeed, an important number of compounds like perfumes, sunscreen components or pharmaceuticals have been reported for their photosensitive properties.
Here, the attention will be focused on halogenated drugs, which are especially known for their ability to induce such disorders. In this context, three compounds have been selected. They correspond to the anti-inflammatory agent carprofen (CP), the platelet antiaggregant triflusal (TF), and the broad spectrum antifungal drug itraconazole (ITZ). Their photochemical and photobiological study has been performed in order to understand the key early events resulting in their reported phototoxicity and photoallergy.
This problem was addressed by a combination of methodologies. In a first stage, photolability of these drugs has been considered and their photoproducts characterized to establish the photoreaction pathways. Then, formation of the excited states generated after light absorption, as well as any other drug-derived short-lived intermediates and/or reactive oxygen species has been determined by spectroscopic techniques as fluorescence/ phosphorescence emission or laser flash photolysis. Finally, photochemical studies have been performed in the presence of biological components to obtain information on interaction between the drug and the biomolecule, photoadduct formation and/or biomolecule oxidation. Overall, this approach has allowed us to evaluate the photobiological risk of the selected compounds.

OC449

New cholesterol-derived probes for solubilization and C-7 radical oxidation studies

Inmaculada Andreu, Fabrizio Palumbo, Edurne Nuin, Miguel Gómez-Mendoza, M. Luisa Marin, Miguel Angel Miranda

[1]*Unidad Mixta de Investigación IISLaFe-UPV, Hospital La Fe, Valencia, Spain;* [2]*Instituto Universitario Mixto de Tecnología Química UPV-CSIC, Valencia, Spain*

Cholesterol (Ch) is an essential component of cell membranes. It has a very limited solubility in aqueous media, and its oxidation via Type I hydrogen abstraction still remains scarcely investigated. These issues have been addressed here by designing new photoactive dyads, whose excited states can be used as probes to monitor incorporation of Ch inside mixed micelles (MM) and the mechanistic aspects of Ch photooxidation via a clean Type I mechanism.
The photoactive dyads have been synthesized by derivatization of Ch at position 3 with dansyl (DNS), 6-methoxy-2-napthyl (MN) and 3-benzoylphenyl (BP) moieties. The DNS derivatives have proven to be efficient tools to monitor incorporation of Ch to MM and to probe the microenvironment experienced inside these entities, which exhibit an outstanding capability to solubilize highly lipophilic compounds.
As regards the BP analogs, they have revealed interesting mechanistic details of the Type I oxidation of Ch via C-7 radicals. The employed analytical tools are steady-state photolysis and laser flash photolysis in combination with quantum mechanical calculations. The topological features of the process are thought to play an important role in Ch oxidation within cell membranes.

OC450

Photodegradation of vitamin B12 in solutions and in humans

Asta Juzeniene, Zivile Nizauskaite

Department of Radiation Biology, Institute for Cancer Research, Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway

Vitamin B12 (cobalamin) is required for proper red blood cell formation, neurologic function, and DNA synthesis. Cobalamins in solutions are light sensitive, but no comprehensive study has been performed to compare the photostability of different cobalamins under UV exposure. Their indirect photodegradation due to their antioxidant properties and their photostability *in vivo* have also not been studied so far.

The photodegradation of methylcobalamin (MeCbl), adenosylcobalamin (AdCbl), hydroxocobalamin (OHCbl) and cyanocobalamin (CNCbl) under UVA exposure in aqueous solutions (pH=7.4) have been investigated by absorption spectroscopy. The photodegradation of OHCbl in the absence and presence of the endogenous photosensitizer riboflavin was studied. Serum vitamin B12 concentrations before and after sunbed use or summer were measured in twenty healthy volunteer or four patients with psoriasis, respectively.

All studied cobalamins are photolabile. The biologically active forms of cobalamin, AdCbl and MeCbl, are converted to OHCbl within seconds during UVA exposure. OHCbl is the most stable cobalamin. However, reactive oxygen species increases the degradation rate of OHCbl. Our pilot study on humans demonstrates that serum vitamin B12 concentrations are not significantly affected during sunbed use or summertime in Norway.

P001

Role of RIP3 in PDT-induced glioblastoma cell death

Grégory Fettweis, Isabelle Coupienne, Marianne Fillet, Jacques Piette

University of Liège, Liège, Belgium

Glioblastoma are the deadliest type of brain cancer. They are associated with poor survival and a high degree of recurrence despite removal by surgical resection and treatment with chemo and radiotherapy. 5-aminolevulinic acid (5-ALA)-based photodynamic therapy (PDT) was recently shown to sensitize human glioblastoma cells (LN18) to programmed necrosis, also called necroptosis. RIP3 (Receptor Interacting Protein 3) kinase, a key factor of the necroptotic signalling pathway, is clearly implicated in PDT-induced glioblastoma cell death. It was shown to associate with RIP1 kinase in a protein complex called necrosome where it autophosphorylates and allows the downstream necroptotic events to take place. Intriguingly, the other factors usually present in the necrosome, namely Caspase-8 and FADD were not encountered in PDT-induced pro-necrotic complex. Our goal is to clarify the composition of the PDT-induced necrosome and to identify the downstream targets of RIP3 to understand the exact mechanism by which 5-ALA-PDT induces cell death in glioblastoma. In this aim, we transduced LN18 cells with 3X FLAG-tagged RIP3 and performed proteomic analysis on the FLAG immunoprecipitate of cells treated by PDT and collected 4 hours post-irradiation. We are currently analyzing the mass spectrometry results and trying to confirm the interactions by co-immunoprecipitation and immunofluroscence colocalization in different human glioblastoma cell lines.

P002

Peptidic Scaffolds for Targeted Delivery of Protease-Sensitive Photosensitizer Prodrugs in Photodynamic Therapy

Nawal Sekkat, Andrej Babic, Norbert Lange

University of Geneva, School of Pharmacy, Laboratory of Pharmaceutical Technology, Geneva, Switzerland

We have previously demonstrated the selective delivery of photosensitizers to prostate cancer through the targeting of proteolytic activity of urokinase-like plasminogen activator (uPA) in vitro and in vivo. Using conceptually new compounds termed as polymeric photosensitizer prodrugs (PPPs) allows for highly selective therapies by targeting multiple pathological disease facets. For instance, the prepared polymeric prodrug conjugates for PDT are designed to sequentially 1) accumulate in pathological tissue (selective extravasation/retention effect through neovascularization), 2) target pathologically related proteases, 3) taken-up selectively by abnormal cells, and 4) being selectively irradiated in the diseased area. Just recently, we have demonstrated the effective treatment of prostate cancer in an experimental animal model.

Although in principle selective and efficient in targeting and treating diseases, in these compounds the photosensitizer moieties in are randomly distributed throughout the polymeric carrier of mostly high polydispersity and observed effects may vary from batch-to-batch.

Here we present a new type of protease sensitive photosensitizer prodrugs based cyclic peptide delivery vehicle. Cyclic peptides with a constrained secondary structure will allow the preparation of prodrugs with distinct molecular weight, in which the number of photosensitizer as well as their position is well defined.

Based on the already tested uPA-sensitive sequence, GSGR/SAG, in these PPPs we first investigated the fundamental designs necessary to obtain template-based photosensitizer prodrugs with suitable quenching/activation characteristics. Then, the influence of the modifications with respect to PEGylation and photosensitizer payload was determined in vitro.

Quenching of PPPs gradually increased with increasing payload from one to four pheophorbide units per peptide. The highest

quenching factor observed was around 200 as compared to a non-quenched probe. In vial, uPA selectively activated all PPPs. Furthermore, activation by this endoprotease was inhibited by the uPA specific inhibitor amelioride. Finally, the uptake and dose response to PDT using uPA-sensitive template based photosensitizer prodrugs was determined in two prostate cancer cell lines showing PPP dose and light dose dependent inactivation of cells.

P003

Can combination of targeted photodynamic therapy with "omics" analysis be the first step to personalized photodynamic therapy?

Andrzej Marcin Bugaj

College of Health, Beauty Care and Education, Poznań, Poland

The increasing cases of cell resistance toward conventional methods of treatment and non-specific toxicity of many drugs on healthy tissues create necessity of the new paradigm of photodynamic therapy (PDT), focused on the concepts of heterogeneity and dynamic state of tumor. Implementation of this paradigm to medical sciences forms the basis for development of new therapeutic strategies, striving for personalized medicine in which medical decisions, practices, and/or products are "tailored" to the individual patient. Combination of targeted photodynamic therapy (TPDT) with analysis of tumor microenvironment using "omics" techniques may be an initial step to personalized PDT.

Targeted photodynamic therapy (TPDT) improves delivery of photosensitizer to cancer tissue and at the same time enhances specificity and efficiency of PDT. Depending on the mechanism of targeting, we can divide the strategies of TPDT into "passive" - using physicochemical parameters of carriers (size, chemical composition, surface properties, electric charge) and pathophysiological differences between healthy and tumor tissue (enhanced permeability and retention effect) to deliver the drug to a target site, "active" - consists in transporting drugs to target cells using specific ligands (loligomers, aptamers) which bind to appropriate receptors expressed at the target site, and "activatable", where the photosensitizer is activated only in the target tissue by enzymes, nucleic acids or environment factors (e.g. pH within tumor cells which is lower than within healthy ones).

Cancer is the phenotypic end point of multiple molecular aberrations and metabolic modifications that have accumulated within its cells. These alterations come together to form complex, dynamic, and plastic networks that govern the "hallmarks of cancer." The development of new technologies and powerful computational algorithms, generally termed as "-omics" methods, have enabled researchers to identify and analyze changes of many biological molecules such as genome ("genomics"), transcripts ("transcriptomics"), proteins ("proteomics") and metabolites ("metabolomics"), and – as a consequence – to identify responsive patient populations, to determine tumor biomarkers, to design clinical trials as well as to implement and use new anticancer drugs.

P004

Effect of spacer length between phthalocyanine and silica gel support upon heterogeneous sensitizer photobactericidal activity

Nina Kuznetsova[1], Olga Yuzhakova[1], Marina Strakhovskaya[2], Ludmila Slivka[1], Oleg Kaliya[1], Evgeny Lukyanets[1]

[1]Organic Intermediates and Dyes Institute, Moscow, Russian Federation; [2]M.V. Lomonosov Moscow State University, Biology Faculty, Biophysics Department, Moscow, Russian Federation

The photodynamic inactivation of microorganisms is a promising approach for disinfection of water. In most studies, for this purpose water-soluble photosensitizers were tested. It was established that photodynamic disinfection of aqueous systems by water-soluble sensitizers is rather efficient. However, removal of water-soluble dyes and their photoproducts from treated solution is extremely difficult. Therefore, the use of heterogeneous photosensitizers where dyes are immobilized onto solid materials might be more promising because heterogeneous photosensitizers can be easily separated from the solutions by centrifugation or filtration.

We have developed new heterogeneous photosensitizers in which positively charged phthalocyanine is anchored to silica by spacers with different lengths. The spectral, fluorescent properties and photosensitizing tendencies of new heterogeneous sensitizers were investigated. The effect of spacer length upon phthalocyanine absorption and fluorescence spectra, singlet oxygen quantum yield was not observed. However efficiency of photodynamic inactivation of bacteria enhanced drastically along with spacer length.

This work was supported by the Russian Foundation for Basic Research (Project No. 13-03-0067).

P005

Cationic antimicrobial peptides as carriers of photo-sensitizers for photodynamic therapy of tumours

Francesca Moret, Cristina Verona, Marina Gobbo, Lucia Celotti, Elena Reddi

Padova University, Padova, Italy

In the last decades, several applications of photodynamic therapy (PDT) have been proposed, including the treatment of cancerous and non-cancerous diseases as well as antimicrobial infections. The therapy is based on the administration of a photosensitizing agent (PS) which, after activation with visible light, exerts cytotoxicity via production of highly reactive oxygen species (ROS) that cause photo-oxidative damages in the cellular sites of PS accumulation and as a consequence cell death. We studied porphyrins and porphyrin-derivatives to explore their potential usefulness for cancer and antimicrobial PDT; in particular we explored strategies for improving the efficiency and the selectivity of PDT in killing cancer cells and bacteria. In this study we evaluate the delivery to cancer cells of PSs conjugated to cationic antimicrobial peptides (CAMPs). CAMPs are known to exert antibacterial activity against selected bacteria but they also exhibit several features typical of cell-penetrating peptides (CPPs) largely studied as carriers of anticancer drugs. Some of the CAMPs appear also to exert selective anticancer activity. Based on this, we are studying CAMPs as new class of carriers for the delivery of PSs for tumour PDT. We are evaluating, in cancer cells *in vitro*, the selectivity of uptake and the efficiency of the delivery of the PS 5(4'-carboxyphenyl)-10,15,20-triphenylporphyrin (cTPP) conjugated to selected CAMPs (Buforin II, Magainin II, Apidaecin). Preliminary experiments in A549 lung epithelial cancer cells revealed that the intracellular delivery of cTPP conjugated to Buforin II or Magainin II was increased about tenfold and thirtyfold, respectively, with respect to the un-conjugated form of the PS, after 5 h of cell incubation. Therefore, when cells were irradiated with 1.5 Jcm^{-2} of blue light, comparable photo-killing efficiencies were measured with the cTPP-conjugates using concentrations at least 10 times lower than those used for the un-conjugated porphyrin. Notwithstanding the greater improvement of PDT outcomes measured in A549 cells using CAMPs-porphyrin conjugates, further investigations are needed in order to determine the interactions of the drug conjugates with non-cancerous cells and to assess that the mechanisms of action of these CPPs in driving the delivery of the PS increases the efficiency and selectivity of the PDT treatment.

P006

Photodynamic inactivation of bacterial and yeast biofilms with a tetracationic porphyrin

Sandra Beirão[1], Sara Fernandes[1], João P. C. Tomé[2], Maria G. P. M. S. Neves[2], Augusto C. Tomé[2], Adelaide Almeida[1], Ângela Cunha[1], Maria A. F. Faustino[2]

[1]*Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal;* [2]*Department of Chemistry & QOPNA, University of Aveiro, Aveiro, Portugal*

The photodynamic inactivation of microorganisms (PDI) is a technique that relies of three non-toxic elements which combined, are able to cause cellular damage and lead to cell death. Cationic porphyrins have been successfully used as photosensitizers (PS) for the inactivation of bacteria, fungi and viruses. However, it is also known that the organization of microorganisms in biofilms confers increased resistance to antimicrobial agents, comparing to that displayed by cells of the same strain in the planktonic form.

In this study, the efficiency of TetraPy+-Me for the PDI of biofilms of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* was evaluated and compared to the efficiency of PDI of these strains in the planktonic form. Mixed biofilms (*S. aureus* + *C. albicans*) were also tested.

Biofilms used in photodynamic inactivation experiments were developed on of 96 well-microplates and the PDI was assessed, after exposure of the biofilms to different concentrations (5, 10 and 20 μM) of PS and irradiation under white light (4 $mWcm^{-2}$) for 30, 60, 90, 180 and 270 min, by the reduction in the concentration of viable cells (colonies forming unit, CFU).

The results show that the PS caused a progressive inactivation of *S. aureus* biofilms throughout the irradiation period. The maximum inactivation (~7 log reduction in CFU concentration) was achieved at the end of the experiment (270 min) in the presence of 20 μM of PS. In biofilms of *P. aeruginosa*, the maximum inactivation corresponded to a 3 log reduction of colony counts. Maximum inactivation occurred with 20 μM of PS and stabilized after 90 min of irradiation. In the biofilm of the yeast *C. albicans* there was a 6 log reduction in the concentration of CFU, observed after 180 min of irradiation for all tested PS concentrations and remaining stable for the rest of the experiments, for all tested PS concentrations. In mixed biofilms, the PDI of *S. aureus* was as efficient as in single-strain biofilms (7 log reduction in CFU) but was less efficient (5 log) for the yeast. In suspended cells, the inactivation factor was identical to that of biofilms for *S. aureus* but, was one order of magnitude higher than in biofilms for *P. aeruginosa* and *C. albicans*.

The results show that the studied cationic porphyrin causes significant inactivation of the microorganisms, either in biofilms or in the planktonic form, and may be regarded as a promising PS for PDI of biofilms, even in cases of bacteria-yeast mixed assemblages.

P007

Development of anti-cancer vaccines by in vitro PDT modulation of cell death: apoptosis, necrosis & autophagy

Lígia Catarina Gomes-da-Silva[1], Luís Borges Rocha[2], Luís Guilherme Arnaut[1,2], Mariette Maria Pereira[1,2]

[1]*Chemistry Department, University of Coimbra, Coimbra, Portugal;* [2]*Luzitin SA, Coimbra, Portugal*

The limited effectiveness of conventional strategies for the treatment of advanced stages of malignancy has been motivating the development of novel anticancer approaches with higher efficacy and less toxicity. PDT is shifting to the center of this arena thanks to its ability to stimulate the host immune system. In this regard, *in vitro* PDT-generated vaccines are believed to be a promising strategy to potentiate systemic PDT. We have synthesized a new generation of bacteriochlorins that absorb at 750 nm and proved to be exceptionally effective in producing ROS and killing cancer cells, being our leading compound designated as LUZ11. The *in vivo* anti-tumor immune response elicited by LUZ11 is now being evaluated. In addition, we are also explored LUZ11 for the *in vitro* development of vaccines for cancer treatment.

It is not yet clear which mechanisms are underlying the anti-tumor immune responses elicited by dying cancer cells nor which cell death pathways are more appropriate to elicit a strong and specific immune response against cancer cells. Further progress in this field can benefit from the ability to control the mechanism of cell death thus, generating homogenous populations of immunogenic apoptotic, necrotic or autophagic cells.

In this work, we showed that with LUZ11, mainly by changing the laser fluence, it is possible to control the mechanism of *in vitro* death of cancer (CT26WT and A549) and endothelial (2H-11) cells. By applying a very low laser fluence (such as ~ 0.145 J/cm^2), cells initiated autophagy (cytoplasm vacuolization and LC3 II labelling), which ended in cell survival or cell death depending on the LUZ11 concentration. Apoptosis is the dominant mechanism for middle laser fluences (as ~ 0.5 J/cm^2) as shown by the chromatin condensation, fragmented DNA, presence of apoptotic bodies and caspases activation. In contrast, dead cells with a typical morphology of necrotic cells were observed within 15 min using high laser fluences such as, ~ 4 J/cm^2.

In vivo combination studies with conventional PDT followed by systemic or peri-tumoral administration of PDT-generated vaccines, formed only by apoptotic or necrotic cells, demonstrated that these combinations increased the overall survival of mice when compared to PDT alone, being the apoptotic cells the ones with the most promissory results. Regarding the route of administration, the best results were attained with the peri-tumoral administration.

P008

Penetration through the placental barrier and embryotoxicity of semiconductor nanoparticles

Vytautas Kulvietis[1,2], Violeta Zalgeviciene[3], Danute Bulotiene[1], Ricardas Rotomskis[1,2]

[1]*Biomedical Physics Laboratory, Institute of Oncology, Vilnius University, Vilnius, Lithuania;* [2]*Biophotonics group of Laser Research Center, Faculty of Physics, Vilnius University, Vilnius, Lithuania;* [3]*Faculty of Medicine, Vilnius University, Vilnius, Lithuania*

We used semiconductor quantum dot (QD) NP to investigate their ability to penetrate placental barrier as these compounds exhibit exceptional optical properties which enable them to be used as fluorescence agents to track NP migration in vivo. We used two types of QD: CdSe/ZnS core/shell QD coated with amphiphilic polymer and polyethylene glycol layer (λ_{fl}=650 nm) and CdTe core QD coated with mercaptopropionic acid (λ_{fl}=630 nm). QD were injected intraperitoneally to the Wistar albino rats on the 13th day of gestation when the placental barrier is completely formed. The pharmacokinetics and biodistribution of QD in the organs was examined using fluorescence spectrometer with fiber optics module up to 24 h after injection. Animal tissues were processed using paraffin embedding for histological examination. Additionally unstained tissue slices were examined under confocal fluorescence microscopy to identify QD localization in the tissues. The embryotoxicity was evaluated by measuring the size and weight of the embryos and teratogenicity was assessed by observing the malformations. The results were compared with classical toxic agent cyclophosphamide.

Our results show that photoluminescence intensity of QD reaches maximum 3 h after intraperitoneal injection and is undetectable after 24 h indicating, that QD are cleared from the circulation. QD distribution was observed in most internal organs with highest accumulation in placenta, thymus, liver. QD appeared in placental tissues, including labyrinth zone, but QD photoluminescence was observed in fetal tissues neither by fluorescence spectroscopy nor by microscopical examination.

Despite of the QD absence in the embryos, maternal exposure to CdTe QD resulted in dose depended embryonic mortality and reduction of size and weight of the fetuses. However QD didn't show teratogenic effects in contrast to cyclophosphamide which caused malformations and embryotoxicity.
CdTe QD underwent spectroscopic changes in vivo - the blue shift and widening of the photoluminescence band - which implies about chemical destabilization of nanocrystal which might be related to the release of cytotoxic core compounds. Meanwhile CdSe/ZnS core/shell QD didn't cause toxic effects and their spectroscopic properties remained stable in vivo. The study shows that QD are unable to penetrate through the placental barrier and they are prevented from accumulation in embryos. However maternal exposure to QD causes embryotoxicity which depends on the type and the dose of NP. The toxic effects of QD might be related to chemical destabilization of NP.

P009

Photodynamic inactivation of bioluminescent *E. coli* by immobilized chlorin derivatives

Mariana Mesquita[1], José C. J. M. D. S. Menezes[1], Maria G P. M. S. Neves[1], José A. S. Cavaleiro[1], A. Cunha[2], A. Almeida[2], Maria A. F. Faustino[1]

[1]Department of Chemistry & QOPNA, University of Aveiro, Aveiro, Portugal; [2]Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal

Photodynamic antimicrobial therapy (PDI) has already been used efficiently to destroy microorganisms in wastewater. This methodology combines a photosensitizer (PS), light and oxygen to achieve selective destruction of microorganisms normally via oxidative damage [1,2]. Porphyrin derivatives and analogues such as chlorins may be considered promising chemical disinfectants for the inactivation of pathogens in wastewater plants, due to the fact that they are effective in inactivating microbial cells without formation of potentially toxic products as occurs in the conventional wastewater treatments [3]. However, the extension of the photodynamic principle to a new environmentally technology can only become viable if the PS is immobilized on a solid matrix in order to allow its complete recovery after the microbial photoinactivation process [2, 4].
The aim of this study was to investigate the use of photoactive materials decorated by cationic chlorin derivatives in the photoinactivation of bioluminescent *Escherichia coli*. The synthesis of the new photosensitizer materials, the photophysical characterization and their antibacterial activity will be presented and discussed.

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P010

Resistance of Oesophageal Carcinoma Cells to Porphyrin-Based Photodynamic Killing can be Overcome by ABCG2 Inhibitors.

Gemma A Barron, Harry Moseley, Julie A Woods
University of Dundee, Dundee, United Kingdom

Adding 5-aminolevulinic acid (ALA) to cells circumvents ALA-synthase, resulting in the production of excess protoporphyrin (PpIX). The accumulated PpIX fluoresces red when irradiated with violet light, and can be used to delineate cancer and dysplastic tissue. Lengthening the wavelength of the irradiating light to ≈ 630 nm, allows sufficient tissue penetration so that the photoactivated PpIX can generate enough cytotoxic singlet oxygen to kill tumour cells (photodynamic therapy, PDT). We studied ALA-induced PpIX synthesis in four human cell types, namely immortalised keratinocytes (HaCaT), bladder carcinoma cells (HT1197), oesophageal carcinoma cells (OE19) and neuroblastoma cells (SHSY5Y). As expected, incubating the cells with 1 mM ALA for 4 hours increased intracellular porphyrins in all cells: HaCaT (10 ± 10 nM porphyrin/10^6 cells); HT1197 (40 ± 10); OE19 (120 ± 50); SHSY5Y (30 ± 10) and HPLC analysis showed that PpIX predominated. Unexpectedly, we found that despite accumulating high levels of intracellular PpIX, OE19 cells were the least sensitive to PDT with 1.5 J/cm^2 red light (HaCaT viability: 71.5 ± 3.3%; HT1197: 31.7 ± 5.5%; OE19: 101.5 ± 9.2%; SHSY5Y: 33.1 ± 10.3%). Confocal microscopy revealed that whereas PpIX fluorescence was diffuse throughout the cytoplasm or concentrated in the plasma membrane of three of the cell types, in OE19 cells it was concentrated in intensely fluorescing intracellular regions. Co-incubating with the ABCG2 inhibitor Ko-143, changed the intracellular distribution of PpIX fluorescence in OE19 cells profoundly, becoming diffuse throughout the cytoplasm. No cell distribution changes were seen in the other cell lines. Moreover, although the inhibitor was effective at increasing the phototoxicity of PpIX in HaCaT and HT1197 cells in a manner related to increased accumulation of PpIX, decreased viability of OE19 cells (to about 30%) was not accompanied by an increase in PpIX accumulation (measured several different ways). Only the redistribution of PpIX observed in the confocal images correlated with the decreased viability of OE19 cells. To conclude, we have identified sequestration of PpIX by OE19 cells which protects them from photodynamic killing despite very high levels of intracellular PpIX. This can be reversed by the ABCG2 inhibitor Ko-143. The sequestration of PpIX by OE19 cells could represent a possible mechanism of reduced sensitivity to PDT, which may be overcome by ABCG2 inhibitors in certain tumours.

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P011

Ex tempore prepared supersaturated solutions of curcumin intended for aPDT

Milica Vukicevic, Anne Bee Hegge, Hanne Hjorth Tønnesen
University of Oslo, School of Pharmacy, Oslo, Norway

A naturally occurring photosensitizer Curcumin (Cur) has already shown good in vitro bacterial phototoxicity.[1,2] However, low aqueous solubility and poor photolytic and hydrolytic stability limit the therapeutic application of Cur in antibacterial photodynamic therapy (aPDT). The concept of supersaturation was explored for enhancing the photodynamic effect of Cur. A supersaturated solution of Cur has already demonstrated phototoxic effect towards bacteria.[2] However, to be useful in the clinical practice the supersaturated solution should have a well defined and sufficient concentration of active ingredient and maintain physical and chemical stability over the relevant time period. Cur was formulated as a solid dispersion (SD) intended for ex tempore preparation of a supersaturated solution. Due to

the transformation of crystalline Cur to amorphous form the desired concentration of Cur could be achieved upon rehydration of SD. The presence of appropriate excipients secured temporal stabilization of the metastable supersaturated solution. A series of SDs with different combinations and ratios of excipients were prepared and studied. UV-Vis spectroscopy was used to examine the extent of supersaturation, HPLC was applied in studies of chemical stability of Cur in the preparations. Finally, a SD with optimal properties was selected for further studies on aPDT effect on bacteria.

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P012

The study of antitumor activity of thioredoxin inhibitors and photodynamic therapy

Angelika Muchowicz[1], Szymon Klossowski[2], Ryszard Ostaszewski[2], Jakub Golab[1]

[1]*Department of Immunology, Center of Biostructure Research, Medical University of Warsaw, Warsaw, Poland;* [2]*Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland*

Thioredoxins are ubiquitous proteins which regulate the activity of a number of signaling pathways and transcription factors. Thioredoxins protect cells against oxidative stress and play a significant role in cell proliferation and inhibition of apoptosis that provide survival advantage to tumor cells. The thioredoxin capacity to reduce other proteins by cysteine thiol-disulfide exchange is essential for protein folding and cellular redox homeostasis. Since photodynamic therapy (PDT) induces oxidative stress, leads to extensive protein oxidation and elevates the amounts of misfolded proteins in tumor cells, we hypothesized that thioredoxin inhibitors could be used to potentiate antitumor activity of PDT. Our results indicate that PDT induces transcriptional up-regulation of thioredoxin and thioredoxin reductase. Overexpression of thioredoxin protects tumor cells against PDT. Furthermore, it seems that thioredoxin participates in the elimination of carbonylated proteins in PDT-treated cells. Thus, we examined cytotoxic activity of the combination of PDT and thioredoxin inhibitors: PX-12 and SK053. PX-12 is an investigational compound evaluated in clinical trials, while SK053 is a novel and originally designed peptidomimetic compound that inhibits the activity of thioredoxin and thioredoxin reductase system. Our results show that both thioredoxin inhibitors effectively sensitize different tumor cell lines to cytotoxic activity of PDT. These compounds activate unfolded protein response and effectively induce apoptosis in tumor cell treated with PDT. Additionally, the antitumor activity of PDT was potentiated by thioredoxin inhibitors in vivo in EMT6 (murine breast carcinoma) and Panc02 (pancreatic carcinoma) tumor models. We conclude that inhibition of thioredoxin-thioredoxin reductase system is a promising therapeutic approach capable of potentiating antitumor effectiveness of PDT.

P013

Photosensitised generation of singlet oxygen in silicone polymer substrates doped with methylene blue for antimicrobial applications.

Melissa Jayne Bovis[1], Josephine Helen Woodhams[1], Sacha Noimark[2], Annapaula Correira[3], Mike Wilson[3], Ivan P Parkin[2], Alexander James MacRobert[1]

[1]*Department of Surgery, University College London, London, United Kingdom;* [2]*Department of Chemistry, University College London, London, United Kingdom;* [3]*Eastman Dental Institute, University College London, London, United Kingdom*

Recent figures suggest that 20 % of all nosocomial infections in the UK are urinary tract infections (UTIs), 80 % of which are associated with urinary catheterisation. Although the cost of catheters and treatment of UTIs is relatively inexpensive, the frequency at which infections occur is costly. The use of silver alloy catheters and antibiotic catheters (minocycline/ rifampicin- or nitrofurazone-coated) has been implemented to reduce infection rates; however, no long-term, sustainable reduction of asymptomatic bacteriuria has been reported. Previous studies at UCL have demonstrated that medical grade silicone catheters coated with the photosensitising dye, methylene blue (MB), can diminish antimicrobial activity following activation at 660 nm. Incorporation of gold (Au) nanoparticles (c. 2 nm diameter) augments bacterial kill. In this study we have investigated the generation of singlet oxygen in 1 mm thick silicone sheets impregnated with MB. The uniformity of the MB distribution within cross-sections of the silicone sheet was validated using fluorescence microscopy. The sheets were placed in distilled water and time-resolved photon counting detection of singlet oxygen phosphorescence at 1270 nm was carried out using pulsed 532 nm laser excitation. Indirect detection was carried out using a hydrophilic chemical trapping reagent (Sensor Green) which becomes fluorescent when it reacts with singlet oxygen. The singlet oxygen lifetime was measured to be 40 microseconds which did not change significantly using lower oxygen tensions, although a slower rise-time of the phosphorescence signal was observed which is ascribed to the reduced rate of quenching of the MB triplet state. The singlet oxygen lifetime is significantly longer than observed in water (c. 3 microseconds) which shows that the silicone matrix does not deactivate singlet oxygen significantly. Studies using Sensor Green present in the surrounding aqueous solution demonstrated that singlet oxygen could be generated. But a signal was only observed using D_2O solution which is consistent with the significantly reduced rate of singlet oxygen quenching in deuterated solvents. We conclude that that singlet oxygen is likely to be a key intermediate in the mechanism of antimicrobial activity of the photosensitised polymer.

P014

The antinecrotic and proapoptotic effect of NO on photosensitized neurons and glial cells

Vera Kovaleva, Elena Berezhnaya, Mikhail Rudkovskii, Anatoly Uzdensky

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

Photodynamic therapy (PDT) is currently used in oncology, particularly, in treatment of brain tumors. We studied the role of NO-mediated signaling in PDT-induced injury and protection of neurons and surrounding glial cells in the crayfish stretch receptor that consists of a single neuron enveloped by glial cells. Alumophthalocyanine photosens (10 nM, 30 min incubation) was used as a photosensitizer; as an irradiation source we used laser diode (670 nm, 0.4 W/cm^2). Application of NO generators sodium nitroprusside (10 μM) and NONOate (10 μM) decreased PDT-induced necrosis of glial cells. NONOate also significantly increased PDT-induced apoptosis of glial cells. Inhibition of neuronal NO-synthase by L-NAME (1 mM) significantly

increased the percentage of necrotic glial cells but did not influence neuronal necrosis. This confirmed the anti-necrotic effect of NO in glial cells and involvement of neuronal NO synthase in their protection. L-NAME (1 mM), L-NNA (1 mM), another inhibitor of neuronal NO-synthase, and inhibitor of inducible NO synthase S-methhylisothioharnstoff sulfate (50 μM) protected glial cells from PDT-induced apoptosis. Therefore NO could be involved in PDT-induced apoptosis of glial cells. Inhibition of NO-activated protein kinase G with 10 μM KT5823 decreased the percentage of necrotic glial cells but not neurons. Therefore, protein kinase G could be involved in PDT-induced necrosis of glial cells, possibly, independently on NO. KT5823 also increased the level of apoptosis of glial cells indicating the anti-apoptotic role of protein kinase G. Thus, NO is involved in regulation of PDT-induced necrosis of neurons and glial cells as well as in apoptosis of glial cells.

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P015

The protection effect of GDNF on photosensitized neurons and glial cells

Maxim Komandirov, Grigory Fedorenko, Anatoly Uzdensky
Southern Federal University, Rostov-on-Don, Russian Federation

Reactions of the nervous tissue to external impacts depend on intercellular neuroglial interactions mediated by neurotrophic factors, which transmit "the survival signals" between cells. We studied the pro-survival effect of glial cell line-derived neurotrophic factor (GDNF, 10 ng/ml) on PDT-induced changes in neuronal activity, ultrastructure and death of neurons and glial cells in the crayfish stretch receptor subjected to photodynamic effect of aluminium phthalocyanine Photosens (10 nM, 30 min incubation) and diode laser light exposure (670 nm, 0.4 W/cm^2). The dual fluorochroming of preparations with propidium iodide and Hoechst 33342 was used for visualization of nuclei of alive, necrotic and apoptotic cells. Photodynamic treatment caused necrosis of neurons and glial cells and apoptosis of glial cells. GDNF significantly decreased PDT-induced necrosis and apoptosis of glial cells. At the ultrastructural level, PDT induced swelling of membranous intracellular organelles: mitochondria, Golgi dictyosomes, and cisterns of endoplasmic reticulum (ER) that led to significant cytoplasm vacuolization. In the presence of GDNF, the ultrastructure of PDT-treated neurons and glial cells was saved and looked the ultrastructure of control samples: the cytoplasm was segregated by Nissl bodies abundant with ribosomes, polysomes, rough ER cisterns, mitochondria, and dictyosomes. Mitochondria saved matrix and well-developed cristae. This indicated maintaining the high level of biosynthetic and bioenergetic processes. The similar effect on glial cells was observed as well. Histochemical study showed that PDT inhibited succinate dehydrogenase in the neuron. However, in the presence of GDNF this inhibition was much less that without it. Thus, GDNF protected neurons and glial cells from PDT-induced damage.

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P016

Susceptibility of non-enveloped DNA- and RNA–type viruses to photodynamic inactivation

Liliana Costa[1], João P. C. Tomé[2], Maria G. P. M. S. Neves[2], Augusto C. Tomé[2], José A. S. Cavaleiro[2], Ângela Cunha[1], Adelaide Almeida[1], Maria A. F. Faustino[2]
[1]Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal; [2]Department of Chemistry & QOPNA, University of Aveiro, Aveiro, Portugal

Antimicrobial photodynamic inactivation (PDI) has been suggested as an alternative approach to inactivate microbial organisms through the use of targeted photosensitizers (PS) and light. Bacteriophage photoinactivation has already been studied under different conditions as surrogates for mammalian viruses. However, there is still scarce information about comparative PDI effect on bacteriophages with different nature of the nucleic acids and capsid proteins (called by DNA and RNA-types phages). The goal of this study was to compare the effect of a tricationic cationic porphyrin on the inactivation of four non-enveloped DNA (T4 like phage, *Aeromonas salmonicida* phage - AS, *Pseudomonas aeruginosa* phage - PA and *Vibrio anguillarum* phage - VA) and three non-enveloped RNA phages (MS2, Q and Si phages). RNA- and DNA-type phages suspensions of 5 x 107 PFU mL^{-1} were exposed to white light (40 W m^{-2}) during 270 min and at concentrations of 0.5 and 5.0 μM of PS, respectively. The photodynamic inactivation showed that DNA- and RNA-type phages were inactivated in the range of 6-7 log, however at different PS concentrations and irradiation times. At the concentration of 5.0 μM of PS reductions of 7 log at 180 min were reached for T4-like phage while the other three DNA-type phages attained 6 log of reduction after 270 min of irradiation. The photodynamic inactivation of RNA-type phages afforded reductions of 7 log at 60 and 90 min, respectively for MS2 and Si, and Q phages, at a concentration of 0.5 μM of PS. The efficacy of photoinactivation varied with the phage nucleic acid type, being RNA-type phages much more easily photoinactivated than DNA-type ones. The RNA-type bacteriophages can be photoinactivate by the tricationic PS to the limits of detection using PS concentration ten times lower and irradiation times four times shorter than the ones required for DNA-type phages. Although both type phages are efficiently inactivated by antimicrobial PDI, the DNA- and RNA–type viruses nature strongly influences the rate of photoinactivation.

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P017

Blood flow and tumor hypoxia following Foscan PDT treatment in a murine model.

Henri-Pierre Lassalle[1,2], Aurélie Reinhard[1,2], François Plénat[1], Sophie Marchal[1,2], François Guillemin[1,2], Lina Bezdetnaya[1,2]
[1]CRAN Laboratory-CNRS-Université de Lorraine, Nancy, France ; [2]Institut de Cancérologie de Lorraine, Nancy, France

In preclinical photodynamic therapy models, pure vascular targeting protocols or pure cellular targeting protocols failed to induce complete and systematic tumor destruction. Several authors then proposed a therapeutic strategy that is a combination of tumor vasculature and neoplastic tissue targeting. We and others demonstrated a substantial improvement of the treatment efficacy up to the complete remission of 100% of the tumors with a dual targeting.

The objective of this in vivo study is to identify which parameters might be predictive of tumor destruction by mTHPC-PDT. Thus, we have been interested in blood flow and intratumoral hypoxia modification under different drug-light intervals (DLI) corresponding to tumor parenchyma, neovascularization or both compartments targeting. Tumor blood flow was measured by a non-intrusive technique of laser doppler.

The intratumoral hypoxia was assessed by using the pimonidazole immunodetection technique. The results were correlated to the photodynamic therapy efficiency for selected DLI.
The results demonstrated a strong reduction in the blood flow, accompanied by an increase in tumor hypoxia for DLI corresponding to vascular localization of the photosensitizer (3h). A transient decrease of the blood flow during PDT treatment was observed for the DLI corresponding to tumor parenchyma targeting, and combined targeting compartments. This transient decrease in flow was not accompanied by changes in tumor hypoxia. The second objective of this study was to determine whether changes in blood flow could predict treatment response. Our results did not underline a parameter corresponding to complete tumor cure.

P018

Optical oxygen sensing in collagen scaffold with porphyrin-based polyacrylamide nanosensors.

Francesca Giuntini[1], Veeren Chauhan[2], Jonathan W. Aylott[2], Geri A. Rosser[3], Alexander J. MacRobert[4], Robert A. Brown[4], Ross W. Boyle[5]

[1]John Moores University, Liverpool, United Kingdom; [2]University of Nottingham, Nottingham, United Kingdom; [3]Durham University, Durham, United Kingdom; [4]University College of London, London, United Kingdom; [5]University of Hull, Hull, United Kingdom

Recent advancements in the fields of scaffold-supported tissue engineering have fuelled a renewed interest in the development of strategies for oxygen measurement and detection. The successful repopulation of cell-seeded matrices/scaffolds and subsequent regeneration of functional tissue depend on the ability to create a favourable environment for cell growth and differentiation, which relies on the constant supply of oxygen and nutrients. The non-uniform regeneration of the extra-cellular matrix (ECM) can prevent adequate oxygen supply to cells and lead to the formation of hypoxic zones in the supporting scaffold, a relatively common cause for the failure of engineered tissues: the measurement of the oxygen diffusivity of a given material is therefore pivotal to assess its suitability to sustain cell growth.
We recently undertook a project aimed at the development of novel porphyrin-based oxygen nanosensors for incorporation in tissue regeneration scaffolds matrices. The possibility of monitoring the global distribution of oxygen within the scaffold during cell growth and migration represents the main advantage of such an approach. Indeed, while the more commonly used fibre optics and electrodes only permit localised measurements, the uniform distribution of an optical probe throughout the matrix of the scaffold allows real-time investigations of the behaviour of the device during the process of recellularization.
We report the synthesis and the oxygen-sensing behaviour of such species, in solution and within doubly-compressed collagen scaffolds, as models for tissue regeneration supports.

P019

Transdermal drug delivery of ALA and Metil-ALA mixtures using human and porcine skin models evaluated by fluorescence spectroscopy and widefield fluorescence imaging

Priscila Fernanda Campos de Menezes[1], Alessandra Keiko Lima Fujita[1], Michelle Barreto Requena[1], Cristina Kurachi[1], Andrigo Barboza de Nardi[2], Andre Escobar[2], Vanderlei S. Bagnato[1]

[1]Instituto de Física de São Carlos (IFSC) - University of São Paulo (USP), São Carlos, Brazil; [2]Faculdade de Ciências Agrárias e Veterinárias - UNESP - Brazil, São Carlos, Brazil

The PDT using ALA and Methyl ALA (M-ALA) have been used in the treatment and diagnostic of different types of cancer and skin diseases. In the topic application using ALA or Methyl - ALA (M-ALA) as precursor of PPIX, there are any limitations. This study has the intention to determine the best mixture using ALA and M-ALA through optical permeation methods. The study was done *in vivo*, using porcine and humans skin models. A 20% ALA cream was applied at a 9 cm^2 and an occlusive dressing placed. PpIX production was monitored using widefield fluorescence imaging and fluorescence spectroscopy collected at skin surface to each hour, until 5h of treatment. The study was developed in 5 pigs and in 5 health humans volunteers, divided in 7 regions in triplicate of mixture (1-7): 100% ALA-0% M-ALA; 80% ALA-20% M-ALA; 60% ALA-40% M-ALA; 40% ALA-60% M-ALA; 20% ALA-80% M-ALA; 0% ALA-100% M-ALA. The porcine skin model is the best skin model to comparing with human skin. However there are any differences related to vascularity since that the human skin still more vascularized than porcine skin. The neonatal porcine skin (less one year old) is more permeable than adult porcine skin. The results to porcine skin using widefield fluorescence imaging, show that, the PPIX production, at superficial layers, is greater and more homogeneous using around 40-60% of ALA mixtures. Also, the same results to human skin were observed. The PPIX formation in deeper layers was greater and faster to mixture of 100% of ALA. However the emulsion with 50% of ALA and M-ALA mixture increases the PPIX production after 5h in deeper layers. The mixture rich in M-ALA for both layers showed lower PPIX production. In this study we prove the similarity of skin models by optical permeation techniques. These results can be useful in Clinical Applications of PDT using topical ALA and M-ALA.
(Financial support: FAPESP).

P020

Cationic galactoporphyrin photosensitisers against UV-B resistant bacteria: oxidation of lipids and proteins by $^1O_2$

Maria C. Gomes, Sandrina Silva, Maria A. F. Faustino, Maria G. P. M. S. Neves, Adelaide Almeida, José A. S. Cavaleiro, João P. C. Tomé, Ângela Cunha

Aveiro University, Aveiro, Portugal

Antimicrobial photodynamic inactivation is becoming a promising alternative to control microbial pathogens. The combination of positively charged groups and carbohydrate moieties with porphyrin derivatives results in an increased cell recognition and water solubility, which improves cell membrane penetration. However, the nature of the oxidative damage and the cellular targets of photodamage are still not clearly identified. This work reports the use of four cationic galactoporphyrins as PS against two environmental bacteria, *Micrococcus* sp. and *Pseudomonas* sp., resistant to oxidative stress induced by UV-B exposure. The effect of $^1O_2$ generated during the PDI assays on oxidation of cellular lipids and proteins was also assessed. PDI experiments with *Micrococcus* sp. and *Pseudomonas* sp. were conducted with 0.5 and 5.0 $\mu molL^{-1}$ of photosensitiser, respectively, under white light at a fluence rate of 150 $mWcm^{-2}$ during 15 min. The most effective compounds against Gram (+) bacteria were PS **3a**, **5a** and **6a** leading to 8.0 log of photoinactivation while PS **3a** and **6a** caused the highest inactivation (6.0 log and 5.3 log) of the Gram (-) strain. The adsorption to cellular material and $^1O_2$ generation capacity of the PS molecule were determinant factors for these inactivation profiles. The occurrence of protein carbonylation and lipid peroxidation supports the hypothesis that antibacterial PDI is triggered by damage of external cell structures such as the cell wall and membrane.
Acknowledgements. *Thanks are due to the Portuguese Foundation for Science and Technology (FCT) and FEDER, for funding the QOPNA (project Pest-C/QUI/UI0062/2011) and CESAM research groups, and the Portuguese National NMR Network. This work had financial support from project PTDC/QUI/65228/2006. M. C. Gomes was financed by the BI grant (BI/UI55/4856/2010) and S. Silva is thankful to FCT for her post-doctoral grant (SFRH/BPD/64812/2009).*

P021

Bacterial nucleic acid as cellular target of photodynamic inactivation by cationic porphyrins

Sónia Coimbra, Maria Bartolomeu, Maria Amparo Faustino, João Tomé, Graça Neves, Augusto Tomé, Ângela Cunha, Newton C. M. Gomes, Adelaide Almeida

University of Aveiro, Aveiro, Portugal

The cellular damages caused by photodynamic treatment have already been studied but the mechanism of bacteria photodynamic inactivation (PDI) is not yet fully understood. Some authors have suggested that although DNA damage occurs, it is not the primary cause of bacterial cell photoinactivation. In the present study we will evaluate the effect of the photodynamic process on the nucleic acids of *Escherichia coli* ATCC 13706 by the two cationic porphyrins Tetra-Py^+-Me and Tri-Py^+-Me-PF.

The two photosensitizers (PS) were used at 5.0 μM upon white light irradiation (4.0 $mWcm^{-2}$). Total nucleic acids were extracted from photosensitized bacteria after different times of irradiation and analyzed by agarose gel electrophoresis. The double-stranded DNA was quantified by spectofluorimetry. The integrity of the *E. coli* cell membrane was evaluated by determination of the release of material absorbing at 260 nm.

In order to evaluate the direct effect of PDI in the nucleic acids, E. coli nucleic acids were extracted, dissolved in TE buffer containing the PS and irradiated. After irradiation, the double-stranded DNA was quantified by spectofluorimetry and the samples were analyzed by electrophoresis. Fluorimetric analysis of DNA unwinding (FADU) was applied to detect DNA strand breaks in the bacterial nucleic acids after PDI. In this communication will be discussed all the experimental details and the main results obtained.

P022

Photoinactivation of *Propionibacterium acnes* with Hypericin

Janice Rodrigues Perussi[1,2], Ana Paula Mazini Calabrese[2], Hidetake Imasato[1,2]

[1]*Instituto de Quimica de São Carlos, São Carlos, Brazil;* [2]*Programa de Pós-Graduação Interunidades em Bioengenharia, Universidade de São Paulo, São Carlos, Brazil*

One of the top challenges in medical dermatology has been the treatment of acne, affecting 80 to 90% of teenagers. The opportunist pathogen *Propionibacterium acnes* are the bacteria responsible for this medical condition, being gram-positive, facultative anaerobic which represents around 50% of the total bacteria of the face. The search for alternative treatment has become important due to bacterial resistance of *P. acnes* to usually applied antibiotics against this etiologic agent and the side effects produced by these drugs. In order to overcome these limitations the photodynamic process could be used to inactivate *P. acnes in vitro*. This technique is based on the combination of a photosensitive molecule, named photosensitizer, oxygen and light. Most of the studies in this field rely on the endogenous synthesis of hematoporphyrin induced by aminolevulinic acid (ALA), a long term treatment using blue light. Hypericin is a good photosensitizer that presents substantial quantum yield, intense absorption spectrum in the visible region, low photobleaching and a large excitation range. The objective of this study was to evaluate the effectiveness of photodynamic inactivation of the *P. acnes* using hypericin irradiated with yellow light. The experiments were performed with a suspension of $1x10^9$ cells mL^{-1} and the microorganism inactivation was achieved even at low concentration of hypericin. The kinetics of intracellular accumulation resulted in a short time of cell intake (around 2.5 min). A delivered light dose of 4.6 J/cm^2 using a Biotable containing yellow LEDs (590 ± 10 nm) was able to reduce 63% of the viable cells using 10 μg/mL of hypericin. The controls showed that hypericin at this concentration and light at this dose were not harmful to *P. acnes* at the experimental conditions. These results show for the first time that hypericin is an effective photosensitizer to photoinactivate *P. acnes.*

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P023

Nucleic acid changes during photodynamic inactivation of bacteria by cationic porphyrins

Eliana Alves, Maria A. F. Faustino, João P. C. Tomé, Maria G. P. M. S. Neves, Augusto C. Tomé, José A. S. Cavaleiro, Ângela Cunha, Newton C. M. Gomes, Adelaide Almeida

University of Aveiro, Aveiro, Portugal

Light activation of photosensitizing dyes in presence of molecular oxygen generates highly cytotoxic reactive oxygen species leading to cell inactivation. Nucleic acids are molecular targets of this photodynamic action but not considered the main cause of cell death. The in vivo effect of the photodynamic process on the intracellular nucleic acid content of *Escherichia coli* and *Staphylococcus warneri* was evaluated herein.

Two cationic porphyrins (Tetra-Py^+-Me and Tri-Py^+-Me-PF) were used to photoinactivate *E. coli* (5.0 μM; 10^8 cells mL^{-1}) and *S. warneri* (0.5 μM; 10^8 cells mL^{-1}) upon white light irradiation at 4.0 $mWcm^{-2}$ for 270 min and 40 min, respectively. Total nucleic acids were extracted from photosensitized bacteria after different times of irradiation and analyzed by agarose gel electrophoresis. The double-stranded DNA was quantified by fluorimetry and the porphyrin binding to bacteria was determined by spectrofluorimetry.

E. coli was completely photoinactivated with both porphyrins (5.0 μM), whereas *S. warneri* was only completely inactivated by Tri-Py^+-Me-PF (0.5 μM). The hierarchy of nucleic acid changes in *E. coli* was in the order: 23S rRNA > 16S rRNA > genomic DNA. The nucleic acids of *S. warneri* were extensively reduced after 5 min with Tri-Py^+-Me-PF but almost unchanged with Tetra-Py^+-Me after 40 min of irradiation. The amount of Tri-Py^+-Me-PF bound to *E. coli* after washing the cells is higher than Tetra-Py^+-Me and the opposite was observed for *S. warneri*. The binding capacity of the photosensitizers is not directly related to the PDI efficiency or nucleic acid reduction and this reduction occurs in parallel with the decrease of surviving cells.

P024

Comparison of Hypericin-PDT with ALA-PDT in U937 and the effect of Hypericin-PDT on U937 cells and HL-60 cells using LED lamps

Nobue Nakajima, Norimichi Kawashima

International Institute for Science and Education, International Pacific University, Yokohama, Japan

Introduction: LED lamps are low cost, easy to operate and reasonably small size. We studied PDT using different photosensitizers and cell lines using LED lamps. We have already studied Hypericin-PDT and determined the incubation time before and after irradiation, the concentration of hypericin and the light intensity of irradiation using a Na-Li lamp and LED lamps in Leukemic monocyte lymphoma cell line (U937). In this experiment, we studied a comparison of Hypericin-PDT with ALA-PDT in U937 cells using LED lamps and the effect of Hypericin-PDT on Human promyelocytic leukemia cells (HL-60).

Methods and Results: U937 cells and HL-60 cells were cultured in RPMI 1640. We prepared the culture solution to provide a final concentration of 5×10^5 cells/ml, and 3ml was added to the culture dishes. 10% of ALA saline solution was prepared. 0.6 μg of ALA was added. Hypericin was dissolved in ethanol to a concentration of 0.5 mg/ml and then we diluted 100 times in saline solution, and 0.5 μg of hypericin was added. We used a LED lamp of 0.31 mW/cm^2 at 633 nm for ALA-PDT and 0.044 mW/cm^2 at 599 nm for Hypericin-PDT. We measured the cell viability after irradiation and after 5 hours of incubation after irradiation. Unfortunately in ALA-PDT, the cell viability was not

decreased to less than 30%, and in addition it increased with further irradiation. In Hypericin-PDT, the cell viability was decreased and nearly 0% after 5 hours of incubation after irradiation in both U937 cells and HL-60 cells.

Conclusion: This study showed that Hypericin-PDT was effective even at a very low light intensity.

P025

Optimization of a multifunctional nanoparticles for applications in photodynamic therapy

Aymeric Seve[1], Hamanou Bénachour[2], Régis Vanderesse[3], Philippe Arnoux[4], Francis Baros[4], Francois Lux[5], Olivier Tillement[5], Muriel Barberi-Heyob[2], Céline Frochot[4]

[1]*LRGP/LCPM, Nancy, France;* [2]*Centre de Recherche en Automatique de Nancy (CRAN), UMR 7039, Vandoeuvre, France;* [3]*Laboratoire de Chimie Physique Macromoléculaire (LCPM) UMR 7568, Nancy-Université, CNRS, Nancy, France;* [4]*Laboratoire Réactions et Génie des Procédés (LRGP) UPR 3349, Nancy-Université, CNRS, Nancy, France;* [5]*Laboratoire de Physico-chimie des matériaux Luminescents (LPCML), UMR 5620, CNRS, Lyon, France*

The strategy developed aims to favor the vascular effect of photodynamic therapy by targeting tumor-associated vascularisation using multifunctional peptide-functionalized nanoparticles. We recently described the design and photophysical characteristics of multifunctional nanoparticles consisting of gadolinium oxide core coated with a layer of silica in which is embedded a photosensitizer (TPC), and a surface-localized tumor vasculature targeting peptide.

In vitro investigations revealed that peptide-functionalized nanoparticles specifically bound to NRP-1 recombinant protein, and conferred photosensitivity to cells over-expressing NRP-1 receptor, demonstrating that the photosensitizer grafted within the nanoparticle matrix can be photo activated to induce cytotoxic effects *in vitro*

In this study, to determine the appropriate settings that will help to obtain the most optimal nanoparticle in terms of selectivity, photodynamic activity and MRI signal intensity, an experimental design has been developed. We will discuss the influence on core size, type of surfactant, concentration of photosensitizer, type and number of peptides grafted onto the photophysical properties, the affinity for neuropilin-1 as well as onto the photodynamic activity of the synthesized nanoparticles.

In perspectives, we could use some specific reactions like Click-Chemistry to perform the elaboration the elaboration of these nanoparticles with possible better results during the synthesis steps.

P026

Overview of Photodynamic Therapy for Polypoidal Choroidal Vasculopathy

Patrycja Nowak-Sliwinska[1], Hubert van den Bergh[1], Adrian HL Koh[2]

[1]*Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland;* [2]*Eye and Retina Surgeons, Camden Medical Centre, Singapore, Singapore*

The first effective therapy for exudative macular degeneration (AMD) was Photodynamic Therapy (PDT). Diagnosis of the disease was to a large extent by fluorescein angiography (FA). Distinguishing between the leaky choroidal neovessels (CNV) associated with classical AMD, and the polypoidal structures associated with Polypoidal Choroidal Vasculopathy (PCV) is not always possible using FA alone. The switch to Indocyanine Green angiography helped to pinpoint PCV, and thus to study the photodynamic therapy of this particular form of retinal disease, which is more frequently encountered in patients in pigmented individuals. The results were quite promising, although in the year following treatment a small fraction of the patients had to be retreated. Alternatively, treating PCV with repeated intravitreal VEGF blocking agents was not as successful as it is in the treatment of wet AMD. Here we discuss the data on PDT of PCV, including combination therapies, alternative treatments, and we also report on similarities and differences between AMD and PCV.

P027

Hypericin-apomyoglobin nanoconstructs for photodynamic therapy applications

Beatriz Rodriguez, Santi Nonell

IQS-School of Engineering, Barcelona, Spain

Hypericin can be assembled at apomyoglobin host scaffold to form a stable 1:1 complex, taking advantage of simple hydrophobic interactions. The nanostructure formation (binding process) has been characterised by a combination of molecular modelling and spectroscopic experiments taking advantage of the high sensitivity of the dye to the microenvironment. A binding equilibrium constant as Ka = (2.4 ± 0.5) × 105 M has been determined. Hypericin fluorescence and singlet oxygen photosensitising properties are preserved in the complex. The kinetic details of singlet oxygen production have been characterised and indicate that protein scaffold shields hypericin from oxygen only to a limited extent. Therefore apomyogobin shows potential as hypericin nanovehicle for theranostic purposes.

P028

Effect of photodynamic therapy (PDT) on immune regulation

Eleonora Reginato[1], Jörg Lindenmann[1], Nina Schweintzger[1], Pablo Augusto Vieyra Garcia[1], Nitesh Shirsath[1], Cord Langner[1], Freyja Smolle-Jüttner[1], Pawel Mroz[2], Michael R Hamblin[3], Peter Wolf[1]

[1]*Medical University of Graz, Graz, Austria;* [2]*Northwestern University, Chicago, United States;* [3]*Harvard Medical School, Boston, United States*

Previous work in experimental models has revealed that the depletion of (immunosuppressive) regulatory T cells (Tregs) can potentiate the efficacy of PDT. We therefore became interested to investigate the immunological changes induced by PDT and the effect of PDT on levels and function of Tregs. Such an effect may be of importance in predicting the effect of PDT in patients with oesophageal squamous cell carcinoma (ESCC), in whom prolonged survival has been reported after multi-treatment approaches.

To investigate this hypothesis blood was collected from patients with invasive ESCC before PDT and 7 and 14 days after treatment. Treg levels in the blood were quantified by FACS and Treg function by co-culture proliferation assays with T effector cells. Our results indicate that PDT significantly reduced by approximately 50% the suppressive capacity of peripheral Tregs from ESCC patients whereas the Treg levels in their blood remained unaffected. This is important since FoxP3 immunohistochemical staining of ESCC biopsies revealed massive infiltration by Tregs within tumor areas, compared to healthy oesophageal mucosa. However, no significant changes in the number of Tregs infiltrating the tumor were seen after PDT.

In parallel, we also investigated the immunological effects of PDT in a mouse tumor model of colon adenocarcinoma (CT26 in BALB/c mice). Our results indicate that PDT transiently increases the level of Tregs in spleen and lymph nodes, reaching a peak at 4 days after PDT. However, this early immunosuppressive effect of PDT seems to be overcome when the therapy is combined with low-dose cyclophosphamide (CY), a treatment that has been proposed to deplete Tregs. We also observed that PDT combined with CY, but not PDT alone, leads to complete tumor regression and in 90% of the cases to permanent tumor remission. To assess memory immunity, were challenged animals cured by PDT and CY with fresh CT26 cells. Surprisingly none of the cured mice rejected the tumors, but

when a second CY administration was given prior tumor rechallenge, 65% of the mice rejected the tumor. This observation suggests that Tregs that had returned after the first depletion by CY were hindering memory T cells, but they could be depleted again by a second administration of CY.
We believe that more effort should be spent in studying the Treg involvement in PDT and a better understanding of the specific immunological events linked to PDT is desirable to improve the treatment strategies and the ultimate outcome in treated patients.

P029

Multiple effects of Sildenafil (Viagra®) on photodynamic treatment of human embrtonic kidney (HEK) cells

Janet Morgan, Yusheng Dong, David Bellnier
Roswell Park Cancer Institute, Buffalo, NY, United States

Photodynamic therapy (PDT) depends on the light activation of a photosensitizer (PS) in the presence of oxygen to treat localized cancer by oxidative reactions caused by photochemical induction of reactive oxygen species (ROS). PDT efficacy is negatively affected by the ABCG2 transporter that pumps substrate PS out of cells and by hypoxia, which limits ROS formation. Inhibiting ABCG2 transport to increase intracellular PS levels and maintaining the oxygen supply during PDT might decrease such resistance. Sildenafil (Viagra®) may help to improve PDT efficacy through several pathways. (1) Nitric oxide (NO) helps maintain the blood supply in tumors to decrease hypoxia by stimulating formation of the 2nd messenger cyclic guanosine monophosphate (cGMP). Sildenafil inhibits the enzyme phospho-diesterase-5 (PDE-5) which prevents breakdown of cGMP, thus prolonging its downstream effects. (2) Sildenafil inhibits ABCG2 transporter activity, by competing for substrate binding sites. (3) Sildenafil also can up-regulate endogenous nitric oxide (NO) by increasing nitric oxide synthase (NOS) expression. However, increased NO may be cytoprotective or cytotoxic, depending on specific tissues and biological conditions. (4) Sildenafil increases the anti-tumor effect of doxorubicin by increasing reactive oxygen species (ROS) production, the primary mediator of a phototoxic response. We hypothesized that sildenafil would enhance PDT with the ABCG2 substrate HPPH (2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a) in human embryonic kidney (HEK293) cells by similar mechanisms. Sildenafil increased cellular HPPH fluorescence in HEK ABCG2+ cells as measured by flow cytometry, which translated into a synergistic effect on HPPH-mediated phototoxicity *in vitro*. Sildenafil alone was minimally cytotoxic only at the highest dose. To determine if any of the phototoxicity was independent of increased HPPH levels and was due to NO effects, HEK ABCG2 negative (PcDNA) cells were tested for phototoxicity and ROS levels in the presence of the NO inhibitor Nω-Nitro-L-arginine (L-NNA). The ROS probe (carboxy-H_2DCFDA) showed increased ROS levels during HPPH-PDT when sildenafil was present which did not decrease in the presence of L-NNA. L-NNA did not inhibit the sildenafil enhanced HPPH-PDT phototoxicity but increased it further. Sildenafil could be used to enhance PDT efficacy of tumors by eliminating resistance due to ABCG2 expressing cells and by increasing ROS in all tumor cells.
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P030

5-aza-dC potentiates antitumor effect of PDT in mouse models through the induction of specific immune response

Malgorzata Wachowska, Magdalena Gabrysiak, Angelika Muchowicz, Joanna Barankiewicz, Tomasz Rygiel, Jakub Golab
Department of Immunology Center of Biostructure Research Medical University of Warsaw, Warsaw, Poland

Photodynamic therapy (PDT) is a clinically approved and minimally invasive solid tumor treatment. It is a two step procedure involving administration and tumor accumulation of a photosensitizer followed by exposure to a visible light. Activated photosensitizer produces cytotoxic reactive oxygen species (mainly singlet oxygen) that result in cellular damage. PDT under some circumstances may lead to development of antitumor immune responses. In most cases PDT alone is insufficient in inducing robust immune response that would lead to complete tumor rejection. Therefore, development of combination therapies that would augment immune-stimulating and antitumor activity of PDT is of critical importance. Epigenetic mechanisms, which involve DNA methylation, act as regulators of gene expression. Aberrant silencing of numerous genes is one of the most frequent molecular changes observed in tumor cells. Epigenetic events play an important role in tumor progression and evasion from immune surveillance. DNA methylation can be modified with chemical agents such as a methyltransferase inhibitor 5-aza-2'-deoxycitidine (5-aza-dC). 5-Aza-dC induces and upregulates numerous genes, such as cancer – testis antigens, including P1A antigen - a mouse homologue of human MAGE family and silenced or downregulated MHC class I molecules. Modifying DNA methylation by 5-aza-dC can potentially change the effectiveness of antitumor immune responses. The aim of this study was to investigate whether 5-aza-dC as an immune-regulating agent can potentiate antitumor effects of PDT in murine tumor models. Incubation of tumor cells with 5-aza-dC led to up-regulation of MHC class I and induction of a P1A antigen expression in EMT6 and CT-26 cells, syngeneic with BALB/c mice, as well as in LLC cells syngeneic with C57BL/6 mice. Combined use of PDT with 5-aza-dC result in stronger antitumor effects when compared to single treatments in EMT6, CT-26 and LLC murine tumor models. Tumor free mice were able to reject re-inoculated tumor cells of the same origin. Depletion of CD8+, but not CD4+, T lymphocytes diminished antitumor effects of the treatment and adoptive transfer of CD8+ from curred mice delayed tumor growth in comparison to control group. Altogether, we observed that combined treatment induces potentiated antitumor effects accompanied by induction of long-lasting and specific immune response. The mechanism responsible for acquired antitumor effect is dependent on the activity of specific CD8+ cytotoxic T lymphocytes.

P031

Preclinical Phototoxicity Assays: Internal Validation using Known Human Phototoxins

Mary E Dougherty, Douglas B Learn
Charles River Laboratories, Horsham, PA, United States

The response of any preclinical model to known positive control materials is a critical step in the safety assessment process and in understanding the ability of any preclinical model to accurately predict the clinical response to a test material. In the absence of a formal validation process, generation of positive control data by the laboratory performing an assay can serve this function. This laboratory undertook two validation studies using a xenon arc solar simulator that permitted exposure with 10 J/cm^2 of UVA, along with a suberythemal UVB dose along with visible light. UVA and UVB doses were expressed as absolute power calculated from data from a US FDA 21 CFR Part 11-compliant spectroradiometer. The female Long Evans (LE) rat and the female hairless albino mouse were the test systems and the known human phototoxins sparfloxacin, ciprofloxacin, 8-methoxypsoralen (8-MOP) and pirfenidone were evaluated, with propranolol as the negative control. Formulations were administered by oral (gavage) daily for three consecutive days and the test systems irradiated under sedation after the third administration. Lightly and darkly pigmented skin sites and the eyes of the LE rat were irradiated, and a single site on the dorsum was irradiated on the hairless mouse. Skin reactions were evaluated twice on the day of exposure and once daily on the three days following exposure. Ophthalmological evaluation of the LE rat eyes three days after exposure and histopathological evaluation of the eyes were performed. As anticipated,

propranolol and the vehicle control did not elicit phototoxicity in either test system. Sparfloxacin elicited dose-dependent cutaneous phototoxicity in both species, with a greater response in the mouse, but no ocular phototoxicity in the rat. 8-MOP elicited cutaneous phototoxicity at lower doses in the rat than the mouse, and ocular phototoxicity responses in the anterior segment. Ciprofloxacin at doses as high as 2000 mg/kg/day in the LE rat and 1000 mg/kg/day in the mouse did not elicit any phototoxicity responses. Pirfenidone at 650 mg/kg/day, the toxic limit in the rat elicited a slight cutaneous phototoxic response while in the mouse it elicited a response at this and 500 mg/kg/day. The differences in the responses of each test system to the phototoxins may in part reflect bioavailability/distribution to the target tissues at the time of exposure. These results demonstrate that understanding the response of a test system to a phototoxin and a UVR source is critical in evaluation of photosafety of test materials, and the results will be discussed in the context of human clinical experience with these phototoxins.

P032

Novel macrocyclic fluorescent rotors exhibiting PDT sensitiser properties

Larisa G. Klapshina[1], Marina Kuimova[2], Irina V. Balalaeva[3], Yakimansky Alexander[4], Maria Angeles Izquierdo[2], Svetlana A. Lermontova[1], Natalia Shilyagina[3], Ilya Grigoryev[1]

[1]*Razuvaev Institute of Organometallic Chemistry, Nizhny Novgorod, Russian Federation;* [2]*Chemistry Department, Imperial College, London, United Kingdom;* [3]*Lobachevsky State University, Nizhny Novgorod, Russian Federation;* [4]*Institute of Macromolecular Compounds of RAS, St. Petersburg, Russian Federation*

Series of novel fluorescent tetra(aryl)tetra(cyano)porphyrazine free bases and their metal complexes were prepared for the potential biomedical applications.

The unique structural feature of these compounds is the alternation of peripheral strongly electron withdrawing and -donor aromatic groups involved into a macrocyclic -electron network (-spacer). Within this class of fluorophores termed fluorescent molecular rotors light induced intramolecular motion dominates the photophysical properties of the dye by changing the population balance between the radiative ("bright") and non-radiative ("dark") exited states. The compounds reported here are found to demonstrate a strong dependence of fluorescence quantum yield (Φ_f) on viscosity (η) following to a power-law relationship which is known as Förster-Hoffman equations

$$\log \Phi_f = Z + X \log\eta$$

where Z and X are solvent and dye –dependent constants

It was shown that these novel dyes combine properties of the fluorescent molecular rotors with photodynamic activity (cell kill upon irradiation with visible light). In prospect that allows monitoring viscosity during real-time photodynamic therapy process. We confirmed that singlet oxygen is indeed generated during the irradiation of the free porphyrazine base with light at 660 nm. Various polymeric nanoparticles incorporating novel chromophores added to the incubation medium containing human epidermal carcinoma A431 cells were investigated by scanning laser confocal microscopy method and lifetime images of porphyrazine base intracellular localization had been obtained. It was established that the maximum uptake of the photosensitizer into a cell was provided by the use of polyimide-*graft*-(polymethacrylic acid) regular polymer brush nanoparticles as the containers for the dye molecules. In this case the essential photosensitizer accumulation in the near-nuclear area had been observed that is very beneficial for an efficient photodynamic therapy. The cell investigations confirmed a significant photodynamic activity of the synthesized compounds

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P033

Photodynamic oxidation of *Staphylococcus warneri* membrane phospholipids: new insights based on lipidomics

Eliana Alves[1], Tânia Melo[2], Cláudia Simões[2], Maria A. F. Faustino[2], João P. C. Tomé[2], José A. S. Cavaleiro[2], Ângela Cunha[1], Newton C. M. Gomes[1], Pedro Domingues[2], M. Rosário M. Domingues[2], Adelaide Almeida[1], Maria Graça PMS Neves[2]

[1]*Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal;* [2]*Department of Chemistry & QOPNA, University of Aveiro, Aveiro, Portugal*

The photodynamic process refers to the combined use of light and a photosensitizer which in the presence of oxygen originates cytotoxic species capable of oxidizing biological molecules, such as lipids. The photodynamic oxidation of membrane phospholipids of *Staphylococcus warneri* by a tricationic porphyrin (5,10,15-tris(1-methylpyridinium-4-yl)-20-(pentafluorophenyl)porphyrin tri-iodide, Tri-Py$^+$-Me-PF) was studied using a lipidomic approach.

S. warneri (10^8 colony forming units mL^{-1}) was irradiated with white light (4 $mWcm^{-2}$, 21.6 Jcm^{-2}) and Tri-Py$^+$-Me-PF (5.0 µM). Non-photosensitized bacteria were used as control (irradiated without porphyrin). After irradiation, total lipids were extracted and separated by thin-layer chromatography (TLC). Isolated fractions of lipid classes were quantified by phosphorus assay and analyzed by mass spectrometry (MS): off-line TLC-ESI-MS, Hydrophilic Interaction Liquid Chromatography (HILIC)-LC-MS and MS/MS. Analysis of the photodynamic oxidation of the corresponding commercial phospholipid standards was also carried out.

The most representative classes of *S. warneri* phospholipids were identified as phosphatidylglycerols (PGs) and cardiolipins (CLs). Lysyl-phosphatidylglycerols (LPGs), phosphatidylethanolamines (PEs), phosphatidylcholines (PCs) and phospatidic acids (PAs) were also identified. After photodynamic treatment, an overall increase in the relative abundance of PGs was observed as well as the appearance of new oxidized species from CLs, including hydroxy and hydroperoxy derivatives. Formation of high amounts of lipid hydroperoxides was confirmed by FOX2 assay. Photodynamic oxidation of phospholipid standards revealed the formation of hydroperoxy and dihydroperoxy derivatives, confirming the observed CL oxidized species in *S. warneri*.

Membrane phospholipids of *S. warneri* are molecular targets of the photoinactivation process induced by Tri-Py$^+$-Me-PF. The overall modification in the relative amount of phospholipids and the formation of lipid hydroxides and hydroperoxides underlies the lethal damage of photosensitized bacterial cells.

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P034

Molecular targeting and dual strategies for photosensitising phthalocyanines. Design and synthetic considerations

Fabienne Dumoulin, Vefa Ahsen

Gebze Instute of Technology, Gebze, Kocaeli, Turkey

The maximum absorption wavelength - centred at 700 nm - and the powerful singlet oxygen generation capability of phthalocyanines make them very attractive as photosensitisers of photodynamic therapy.

Having explored the different ways to make them water-soluble [1], we prospected several strategies to develop phthalocyanine-based photosensitisers of third generation.

With a focus on the design and on inherent synthetic considerations, the conjugation of potentially targeting moieties - carbohydrates, vitamins - and of chalcones [2] to induce a dual antivascular and photodynamic effect will be presented.

[1] F. Dumoulin, M.Durmus, V. Ahsen and T. Nyokong, Synthetic pathways to water-soluble phthalocyanines, Coord. Chem. Rev. 2010, 254, 2792-2847
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P035

Evaluation of the interplay among the charge of porphyrinic photosensitizers, lipid oxidation and photoinactivation effectiveness in *Escherichia coli*

Diana Lopes[1], Liliana Rosa[1], Maria A. F. Faustino[2], João P. C. Tomé[2], Augusto C. Tomé[2], José A. S. Cavaleiro[2], Ângela Cunha[1], Pedro Domingues[2], M. Rosário M. Domingues[2], Adelaide Almeida[1], M. Graça P. M. S. Neves[2]

[1]*Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal;* [2]*Department of Chemistry & QOPNA, University of Aveiro, Aveiro, Portugal*

Photodynamic inactivation (PDI) is a simple and controllable method to destroy microorganisms based on the production of reactive oxygen species (ROS) (*e.g.* free radicals and singlet oxygen), which irreversibly oxidize microorganism vital constituents resulting in lethal damage. This technology requires the combined action of oxygen, light and a sensitizer (PS), which absorbs and uses the energy from light to produce those ROS. The main targets of the antibacterial photodynamic activity are the external microbial structures, such as cell walls and cell membranes. For a better understanding of photoinactivation process, the knowledge of how some molecular targets are affected by PDI assumes a great importance. The aim of this work was to study the effect caused by a series of cationic porphyrin derivatives, bearing one to four positive charge, used as PSs, during the photoinactivation process on *Escherichia coli*, bacterial lipids. In this context, the effect of five porphyrin derivatives used to inactivate *E. coli* was evaluated by the quantification of lipid hydroperoxides and by analysis of the variation of fatty acyl profiling. *E. coli* suspensions were irradiated with white light in the presence of each PS (5.0 µM) and non-photosensitized bacteria were used as dark (with PS and without light) and light (irradiated without PS) controls. After PDI, the total lipids were extracted and quantified by phosphorus assay and the fatty acyl profiling analysis was done by gas chromatography (GC). After PDI, was observed an overall increase in the lipid hydroperoxides contents depending to the PS charge and its distribution on the macrocycle. The pattern of lipid oxidation has a high correlation with photoinactivation effectiveness of the five cationic porphyrins. In fact the PS that induced higher lipid oxidation is the one that corresponded to higher bacterial inactivation. Analysis of fatty acyl profile by GC showed a decrease in the unsaturated fatty acids, corroborate the relationship between lipid oxidation and photoinactivation. It can be concluded that bacterial membrane phospholipids are important molecular targets of photoinactivation being the formation of hydroperoxy derivatives a good indicator of the process.

P036

Hypericin-aPDT Treatment of Experimental Periodontitis *in vivo*

Livia Rodrigues Perussi Valverde[1], Rafael Scaf de Molon[1], Guilherme José Pimentel Lopes de Oliveira[1], Anderson Orzari Ribeiro[2], Janice Rodrigues Perussi[3], Rosemary Adriana Chierici Marcantonio[1]

[1]*Faculdade de Odontologia de Araraquara, UNESP - Brasil, Araraquara, Brazil;* [2]*Centro de Ciências Naturais e Humanas, UFABC - Brazil, Santo André, Brazil;* [3]*Instituto de Química de São Carlos, USP - Brazil, São Carlos, Brazil*

Antimicrobial photodynamic therapy (aPDT) has been proposed as an auxiliary treatment of periodontitis. Periodontal diseases involves accumulation of oral biofilm and its progression leads to bone loss, one of the most common cause of tooth loss. Hypericin (HY) is a very potent photoactive natural pigment. The aim of this study was evaluate the effects of HY-aPDT in the treatment of periodontitis induced in vivo. Thus, 84 rats were submitted to periodontitis induction by placing ligatures around superior 2nd molars. After 7 days, ligatures were removed and animals were randomly distributed in 4 groups: Group I – Control, disease without treatment; Group II – SRP, scaling and root planning with manual periodontal curettes; Group III – aPDT, application of HY (10 µg/ml) for 5 minutes in the periodontal pocket followed by 4 minutes of 630 nm irradiation per tooth (35,15 J/cm^2); Group IV - SRP+aPDT, association of the treatments of groups II and III. Seven, 15 and 30 days post-treatment the animals were sacrificed. MicroCT tridimensional evaluation and histometric analysis showed statistical differences between Control and treatments groups at 7 and 15-days. Therefore, HY-aPDT was able to reduce periodontitis progression in vivo in a similar way to mechanical treatment, considered the conventional treatment of the disease.

P037

Photolon(R) - from pilot experiments to routine clinical practice

Tatsiana Trukhachova[1], Siarhei Shliakhtsin[1], Yuri Istomin[2], Mikhail Kaplan[3], Aleksandr Fedulov[4], Liudmila Marchanka[4]

[1]*RUE "Belmedpreparaty", Minsk, Belarus;* [2]*N.N. Alexandrov National Cancer Centre of Belarus, Minsk, Belarus;* [3]*Medical Radiological Research Center of Russian Academy of Medical Sciences, Obninsk, Russian Federation;* [4]*Belarussian State Medical University, Minsk, Belarus*

Long-term basic research in photochemistry, photophysics and photobiology of chlorin e6 led to the development of the first chlorin e6-based photosensitizer (PS) Photolon®, approved in Belarus in 2001 and in Russian Federation in 2004.

Here we summarize the spectral and photophysical properties of Photolon®, its interaction with tumor cells, *in vivo* experimentation, including the biodistribution of the PS in healthy and tumor-bearing animals, PS pharmacokinetics upon different routes of administration (i.v., oral, topical), the maximum tolerant and toxic doses of Photolon® in different species, and mutagenic, teratogenic and embryotoxic activity of PS.

Within a number of Photolon® clinical trials a convincing evidence of its therapeutic efficacy in patients with skin and mucosal malignancies (including disseminated forms of melanoma), central lung cancer, cervical intraepithelial neoplasia, primary and metastatic brain tumors, tumors of the oral cavity, bladder cancer and macular degeneration was obtained.

High therapeutic efficacy and good tolerance of Photolon® in various categories of patients and existing 10-year clinical experience provide new possibilities for wide introduction of PDT with Photolon® into the clinical practice for the treatment of various cancers and precancerous diseases.

P038

Synthetic strategies for chalcone-porphyrin conjugates

Derya Topkaya Taşkıran[1], Sinem Tuncel[2], Emel Önal[2], Khalil Bennis[3], Catherine Hirel[2], Vefa Ahsen[2], Sylvie Ducki[3], Fabienne Dumoulin[2]

[1]Dokuz Eylül Üniversitesi, Izmir, Turkey; [2]Gebze Institute of Technology, Gebze, Kocaeli, Turkey; [3]Clermont Université, Clermont-Ferrand, France

Chalcones are vascular disrupting agents (VDA) which efficiently destroy tumour neovasculature.[1] In order to combine photodynamic and vascular disrupting effects, chalcones have already been linked to photosensitizing phthalocyanines.[2]

We report here the conjugation of different chalcones to photosensitizing porphyrins conveniently designed and functionalized.

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[2] S. Tuncel, J. Fournier-dit-Chabert, F. Albrieux, V. Ahsen, S. Ducki and F. Dumoulin, Towards dual photodynamic and antiangiogenic agents: design and synthesis of a phthalocyanine-chalcone conjugate, *Org. Biomol. Chem.* 2012, *10*, 1154-1157

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P039

***H. pylori* inactivation and virulence gene damage using a Ru(II) sensitiser supported on glass microparticles for photodynamic therapy**

Miryam Calvino-Fernández[1,2], Selma Benito-Martínez[1], Xavier Calvet[2,3], Adrian Gerald McNicholl[2,4], Trinidad Parra-Cid[1,2], Javier P. Gisbert[2,4], David García-Fresnadillo[5]

[1]Unidad de Investigación, Hospital Universitario de Guadalajara, Guadalajara, Spain; [2]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain; [3]Hospital de Sabadell, Barcelona, Spain; [4]Hospital Universitario de La Princesa and Instituto de Investigación Sanitaria La Princesa, Madrid, Spain; [5]Dpt. Organic Chemistry, Faculty of Chemistry, Complutense University, Madrid, Spain

About half of the world's population is currently infected with *H. pylori*, which is involved in the development of several gastro-duodenal pathologies. The increasing number of antibiotic resistance reduces the effectiveness of the first-line therapy, so new strategies to improve the *H. pylori* eradication rates are needed. Antimicrobial Photodynamic Therapy (APDT) benefits from photogenerated reactive oxygen species, such as singlet oxygen, which inactivate microorganisms by means of photosensitizing dyes and visible light. Therefore, it could be a suitable alternative for *H. pylori* eradication in the gastro-duodenal tract, particularly in patients infected with antibiotic resistant strains.

We have evaluated APDT against *H. pylori*, *in vitro*, using a new photosensitizing material (PSM) based on a ruthenium(II) complex tris[(1,10-phenanthroline-4,7-diyl)bis-(benzene-sulfonate)]rutenate(II) as a singlet oxygen photosensitizer (Φ_Δ 0.43 in water), covalently bound to micrometric glass beads (Ø 150–212 μm) via sulfonamide bonds. The dye concentration is 0.34 mg of Ru(II) complex per 100 mg of photosensitizing material.

Five *H. pylori* isolates (classified according to *cagA* genotype, and metronidazole-clarithromycin resistance) were used. Bacteria were mixed with the PSM in 96-well plates and incubated in the dark or illuminated by blue light (LED lamp λ_{em}^{max} 465 nm, fluence rate E_0 14.1 ± 0. 7 mWcm^{-2}). Aliquots were cultured and colonies were counted after 2–3 days. A 95% decrease was detected in the number of colonies in the irradiated wells where the bacterium was mixed with the PSM, compared to non-illuminated wells or with irradiated wells without PSM. It was also confirmed that DNA is a molecular target for oxidant species released during APDT (evaluated by alkaline gel electrophoresis after endonuclease III incubation, *ureC* and *cagA* RT-PCR, and bacterial fingerprint). Results were independent of *cagA* gene and antibiotic resistances.

P040

Pheophorbide-a delivery by copolymer micelles for photodynamic Therapy: uptake, subcellular localisation and phototoxic activity.

Rachid Kerdous[1], Laure Gibot[2], Jean Pierre Souchard[3], Jerome Ehrhart[3], Anne-Françoise Mingotaud[3], Marie Pierre Rols[2], Stéphanie Bonneau[1], Patricia Vicendo[3]

[1]Laboratoire Jean Perrin, Paris, France; [2]Institut de Pharmacologie et Biologie Structurale, Toulouse, France; [3]Laboratoire des IMRCP, UMR 5623, Toulouse, France

Photodynamic therapy (PDT) is based on the combined action of a photosensitizer (PS), light and oxygen. After the light irradiation, the photo-oxidation reactions lead to the formation of cytotoxic reactive oxygen species inducing tumor eradication. In this context, we were interested in studying pheophorbide-a, a particularly promising PS for PDT because of its good singlet oxygen quantum yield and its photocytotoxic activity. However, the therapeutic activity of this hydrophobic compound can be improved by increasing its bioavailability and secondly its capture by the reticuloendocytic system. To address these limiting factors, nanoparticles were used. We solubilized pheophorbide a in poly (ethylene oxide-b-ε-caprolactone) (PEO-PCL) micelles. The nanoparticles containing the PS have an average size of 20 nm. They were characterized by light scattering, electron microscopy, AFM. This strategy increases by a factor of ten the cellular uptake of the PS and its photocytotoxic activity. The perinuclear subcellular localization of PS is not affected by the nanoparticles. The use of copolymer micelles labelled with fluorescein to explore the mechanism of the nanoparticles entrance into cells. The original results of these studies will be presented.

P041

Cytotoxic effects of Pc 4 Photodynamic Therapy on *Trichophyton rubrum*

Elma Baron, Minh Lam, Matthew Dimaano, Pranab Mukherjee, Mahmoud Ghannoum

Case Western Reserve University, Cleveland, United States

Dermatophytes are the most common superficial fungal infections of the skin, hair and nails. Terbinafine remains the main therapy but terbinafine-resistance is emerging. Photodynamic therapy has been employed in the treatment of various infections. Our group published our study on the effects of silicon phthalocyanine Pc 4 photodynamic therapy on *Candida albicans* in vitro. We then sought to determine whether Pc 4 PDT was likewise effective against *Trichophyton* species. Cultures of *Trichophyton rubrum* were incubated with Pc 4 solution and examined under confocal microscopy to evaluate Pc 4 penetration into the fungal cells. Confocal imaging confirmed that after minutes of exposure to Pc 4, *T. rubrum* cells incorporated Pc 4 into their cytosolic compartment. Pc 4-PDT was then performed using 1 micromolar Pc 4 solution followed by 2 J/cm^2 of 675 nm light. Metabolic assays indicate loss of activity after Pc 4-PDT. Clonogenic assays demonstrate cytotoxicity after Pc 4-PDT in both terbinafine sensitive and resistant strains. These suggest that Pc 4-PDT may be developed as a therapy for dermatophytic infections.

P042

Induction of beta-catenin signaling in response to UVB irradiation in melanocytes

Valerie Petit, Irina Berlin, Juliette Bertrand, Lionel Larue

Institut Curie, Orsay, France

Excessive exposure to ultraviolet (UV) rays may lead to the transformation of melanocytes into melanoma, the most aggressive type of skin cancer. For long time, it has been thought that UV were the principal driver for melanoma initiation, however recent deep-sequencing of melanoma samples show that the incidence of UV-induced mutations are much less than expected. On the other hand, one third of melanoma cell lines and primary melanomas have been shown to overproduce nuclear beta-catenin, suggesting a role for beta-catenin in melanoma genesis. We therefore decided to evaluate the molecular mechanisms associated with UV-induced melanocytes transformation and investigate the effect of UV irradiation on beta-catenin signalling in melanocytes. As expected, the irradiation of melanocyte or melanoma cells by UVB induces a rapid phosphorylation of p38 kinase. This kinase is able to phosphorylate and inactivate GSK3, leading to an increase of the stabilized beta-catenin pool. As a consequence, beta-catenin translocates into the nucleus in response to UV irradiation. These results were confirmed in vivo; mouse pups were UVB irradiated and we observed that the translocation of beta-catenin into the nucleus in the upper layer of the skin. The UV-dependent activation of beta-catenin signalling was characterized by an increase in the melanin content of melanocytes, highlighting the key role of beta-catenin in mediating the defensive pigmentation of melanocytes in response to UV stress.

P043

Singlet oxygen production of vitamins under UV radiation

Johannes Regensburger, Alena Knak, Anita Gollmer, Tim Maisch, Wolfgang Baeumler

Department of Dermatology, University of Regensburg, Regensburg, Germany

Vitamins are essential for a good healthy human body and need to be taken up with the diet. A lot of endogenous vitamins, which are settled in human cells, can absorb UVB radiation and could be endogenous photosensitizers. It is known today that the very oxidative reactive oxygen species singlet oxygen cannot only be generated by UVA excitation but also by UVB excitation in a photosensitized process. In addition UVB irradiation leads often to altered molecules with a different capability to generate singlet oxygen.

The aim of the study was to investigate whether and to which extent UVB and/or UVA excitation of endogenous vitamins can lead to the formation of singlet oxygen. Also the singlet oxygen generation of endogenous vitamins can change while irradiated, which needs to be explored as well.

Potential endogenous vitamins were irradiated in solution using monochromatic UVB (308 nm) or UVA (330, 370 nm) radiation. Singlet oxygen was directly detected and quantified by its luminescence at 1270 nm. The alteration of the vitamins while irradiated was determined by changes in the absorption spectra. The singlet oxygen generation of altered vitamins was investigated as well by detecting directly the luminescence at 1270 nm.

All investigated vitamins showed distinct singlet oxygen luminescence signals with quantum yields ranging from around 1 to 64 %. UVB altered the vitamins during irradiation causing a change of absorption in the UV spectrum (280 - 400 nm). For some vitamins an altered efficiency of singlet oxygen generation could be detected.

P044

Cyclized products of thermal decomposition of human eye UV filters: could they be potential photo-initiators of oxidative stress?

Peter Sergeevich Sherin[1], Ekaterina Anatolevna Zelentsova[1], Vadin Vladimirovich Yanshole[1], Olga Aleksandrovna Snytnikova[1], Eric Vauthey[2], Yuri Pavlovich Tsentalovich[1]

[1]International Tomography Center SB RAS, Novosibirsk, Russian Federation; [2]University of Geneva, Geneva, Switzerland

The lens of human eye contains low-molecular weight compounds, absorbing in the region 300-400 nm and protecting eye tissues from harmful UV irradiation. The UV filter functionality is provided by an ultrafast and highly efficient conversion of the absorbed photon energy into heat. Thermal instability of UV filters results in the formation of different products, among them are cyclized compounds such as xanthurenic acid (XAN), kynurenine yellow (KNY), kynurenic acid (KNA) and 4-hydroxyquinoline (4HQN). The concentrations of these products in the human lens are below the detectable level that probably points to their higher chemical and photochemical activity respectively to the parent UV filters.

The main goal of the present work is to study the photophysics and photochemistry XAN, KNY, KNA and 4HQN in neutral aqueous solutions. Our results demonstrate that the photolysis of each system results in the formation of reactive species (of different nature for each molecule), which could be potential initiators of oxidative stress– the main causative factor for the cataract development. Steady-state and time-resolved optical spectroscopy was the main tool of current work.

The excitation of XAN by a laser pulse results in the ultrafast decay of excited states via solvent-assisted tautomeric transformations with the restoration of the initial keto form through the formation of an intermediate enol form in the ground state. The high photochemical stability of XAN indicates a low reactivity of the enol form towards to the initial substrate; however it can initiate the photooxidation of proteins [J.E. Roberts et al., Photochem. Photobiol., 75 (2001) 740].

The main decay channel of KNY singlet excited state is a fast radiationless S_1 ---> S_0 transition. However, the photolysis of KNY also results in the formation of two unstable intermediates: the triplet state and the enol form with yields of about 2% and 1%, which rapidly decay with the formation of 2,3-dihydro-4-hydroxyquinolinone and 4HQN, respectively.

Under UV irradiation, both KNA and 4HQN demonstrate the formation of long-lived triplet state with yields of about 35 and 80%, respectively. This triplet state can react with the aromatic amino acids, tryptophan and tyrosine, and the antioxidant ascorbate via electron transfer mechanism from substrate to the photoexcited molecule.

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P045

The effect of UVA radiation on the topical application of benzo[*c*]phenathridine alkaloid sanguinarine

Jitka Vostálová[1], Pavel Kosina[1], Lucie Novotná[1], Bohumil Zálešák[2], Jitka Ulrichová[1]

[1]Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic; [2]Department of Plastic and Aesthetic Surgery, University Hospital Olomouc, Olomouc, Czech Republic

The natural substances possess a wide range of biological activities and therefore they are presented as active components in dermatological and cosmetic products. Their application is usually based on their use in traditional medicine.

Sanguinarine (SG), a benzo[*c*]phenanthridine alkaloid, is a constituent of some dermatological preparations due to its anti-inflammatory and antimicrobial activities. However, the safety of topical application of SG is unknown to date. The aim of this study was to assess the cytotoxicity, phototoxicity, skin cell biovailability and transdermal transport of SG.

It was found that SG is toxic to human dermal fibroblasts (IC_{50} = 1.94 μmol/l) and the cells transformed SG to the less toxic dihydrosanguinarine (DHSG, IC_{50} > 50 μmol/l), as was similarly described in hepatocytes. After UVA exposure, the IC_{50} value of DHSG/SG changed to 0.625/0.865 μmol/l, respectively. The increase of UVA-induced toxicity was higher for DHSG. HPLC/MS analysis found that UVA irradiation induced the back conversion of the less toxic metabolite DHSG to toxic SG. The conversion is accompanied by overproduction of reactive oxygen species that are responsible for damage of biomolecules, including DNA single-strand breaks formation. SG and DHSG accumulated only in the *stratum corneum* layer of the epidermis and did not penetrate to the deeper layers of the skin, as observed *in vitro* using Franz diffusion cell method with human skin. Higher levels of the alkaloids were detected in *s. corneum* of frozen skin than in that of fresh skin. The amount of DHSG transformed from SG in fresh human skin tissue was smaller than in fibroblast cell cultures. Therefore we assume that SG does not permeate through the epidermis to the deeper layers of metabolic active skin cells.

Our results suggest that topical application of the alkaloid SG and its metabolite DHSG may be associated with modulation of their toxicity by UVA.

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P046

Oxidative stress and metabolism detoxication equipment in normal human melanocyte

Laurence Denat, Christophe Jones, Philippe Perez, Jean-Philippe Belaidi, Laurent Marrot

L'OREAL Research & Innovation, Advanced Research, Aulnay-sous-Bois, France, Paris, France

Melanin has been described as a double-edged sword because of its ability to both generate and scavenge reactive species. Melanocyte has thus to cope with various sources of oxidative stress, especially when exposed to sunlight and it should have got specific endogenous defences. Here we report data linking melanin content and oxidative stress in normal human epidermal melanocytes in culture. In normal human Caucasian melanocytes in culture, oxidative stress induced by UV radiation from a solar simulator increased when melanogenesis was stimulated. Moreover photoinduced DNA damage detected by comet assays and fluorescence of a specific probe used to assess endogenous pro-oxidant status were enhanced upon UV exposure when melanogenesis was triggered. In order to get more information at the molecular level, the antioxidant defences of melanocytes were studied and compared with keratinocytes, either at the basal level or under stress. In a melanocytes/keratinocytes coculture it appeared that expression of some Nrf2 downstream antioxidant genes such as Heme Oxygenase 1 (HO1), NADPH-Quinone-Oxidoreductase 1 (NQO1), and Ferritin as well as glutathione content clearly differed in melanocytes and keratinocytes from the same donor at basal level. After treatment by H_2O_2 or UV, these genes were also differently regulated in melanocytes and keratinocytes. In conclusion, specific physiological antioxidant defences are expressed by melanocytes to protect this cell type against oxidative stress. Further studies have to be conducted in order to better understand how melanocytes manage to cope with this oxidative stress.

P047

Prevalence of actinic keratoses among dermatologic outpatients in Austria

Johanna Eder, Knut Prillinger, Franz Trautinger

Karl Landsteiner Institute of Dermatological Research, St. Poelten, Austria

Background: Actinic keratoses (AK) as common UV-induced precursor lesions of squamous cell carcinomas (SCCs) are an important public health issue with data on their prevalence in Europe largely lacking.

Objective: To define the prevalence of AK according to age, sex and body-site in dermatologic outpatients in Austria to better identify the target population for AK-screening, treatment and prevention.

Methods: In this prospective study 49 office-based Austrian dermatologists were randomly selected to record the presence, number and sites of AK together with age and sex in 100 consecutive outpatients aged above 30 years irrespective of the reason for the office visit. Randomization was done by state and population density to guarantee equal coverage of the federal territory. All data were collected within one month (October 2011) and evaluated with descriptive statistics.

Results: Data on a total of 4449 patients (mean age 61; 55% female, 45 male) were collected 1376 (31 %) of which had AK. 43 % of all AK-patients were female and 57 % were male with a mean age of 74 in both sex groups. AK prevalence increased with age from 2 % (age group 30-39) to 68 % in females and 90 % in males (age > 90). AK were diagnosed most frequently on the center of the head (n=1035; 607 males, 428 females) with the number affected body-sites rising with patients' age. A high density of AK (more than five per site) was observed in 13 % of males 4 % of females with the center of the head being the most frequently affected site. Patients living outside of Vienna had a higher number of simultaneously affected sites (2.9 +/- 2.1) compared with urban residents (2.0 +/- 1.3).

Conclusions: Our results for the first time provide a reliable estimate of the prevalence of AK in dermatologic outpatients in Austria. About a third of all patients are affected, with disease rates rising with increasing age. Screening for AK through office-based dermatologists followed by counselling on appropriate UV-protection and treatment should be further investigated for their potential for the prevention of invasive SCC.

P048

The contribution of Solar and sunbed exposure to skin cancer risk. It all adds up!

Patrick Tierney[1], Frank R. de Gruijl[2], Sally H Ibbotson[1], Harry Moseley[1]

[1]The Photobiology Unit, Ninewells Hospital and Medical School, University of Dundee, Dundee, United Kingdom; [2]Leiden University Medical Center, Leiden, Netherlands

Solar Ultraviolet (UV) radiation is acknowledged as the principle cause of skin cancer. Furthermore, sunbeds have been classified as carcinogenic by International Agency for Research on Cancer.[1] Therefore an increased risk of developing non-melanoma skin cancer (NMSC) is expected when one is exposed to both sources of UV radiation. Following a comprehensive study of 402 sunbeds, the irradiance at the surface of the skin was calculated for a typical sunbed session. It was discovered that 90% of the tanning units were above the recommended 0.3 W/m^2 erythemal effective irradiance level.[2] Application of the SCUP-h tumour-weighting factor[3] showed that the average artificial tanning unit has a carcinogenic risk per minute of exposure that is 2.3 times that of midday Mediterranean sun.

A risk model was derived to include age at first sunbed exposure, number of sunbed sessions and annual holiday patterns. Sunbed session practice of pre-holiday 'base' tanning, moderate (20 sessions per year) to extreme three times a week exposure were also incorporated into the model. We additionally investigated

the various body-sites, from those normally exposed such as face and arms to more usually unexposed sites. The latter include the trunk and legs which account for a large body surface area of ~80%. We calculated the mean total erythemal irradiance to be 0.56 ± 0.21 W/m^2 which is equivalent to 3.36 SED (standard erythema dose) for a ten minute sunbed session. The additional cancer risk was determined by adding this whole body dose to the median annual UV dose of 166 SED which is taken from the Danish cohort.[4] For a moderate 20 sessions per annum the cumulative incidence was calculated to be 1.42 up to age 65 years old. The resultant additional risk incurred by a sunbed user was 1.92 times that of a non-user. This is an estimate of the relative risk of having contracted a SCC at age 65 years old. Risk of developing a NMSCs increases rapidly with more sunbed sessions.

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P049

The effect of sunlight on the Vitamin D pathway

Elke Hacker[1], Leesa Wockner[2], Thomas Pollak[2], Nicholas Hayward[2], David Whiteman[2], Michael Kimlin[1]

[1]QUT, Brisbane, Qld, Australia; [2]QIMR, Brisbane, Qld, Australia

Several studies have established an association between higher 25-hydroxyvitamin D3 levels, at diagnosis and thinner tumours and better survival from melanoma. Understanding the role of vitamin D in cutaneous health and the impact of sun exposure on these pathways requires further investigation. We undertook a clinical trial to compare the molecular regulation of the vitamin D pathway in human skin following solar simulated ultraviolet radiation (SSUVR). We recruited 57 Caucasian participants and exposed several small areas of their lower back to a mildly burning dose of SSUVR with sunscreen applied to one site for comparison. Biopsies were taken from these sites at time-points following ultraviolet radiation exposure and immuno-histochemistry was used to assess molecular changes in the vitamin D pathway. We will present our preliminary findings from our clinical study exploring whether the response of the vitamin D pathway to SS-UVR *in vivo* is modified by genotype, host phenotype or sunscreen.

P050

Photochemical and microbial alterations of DOM spectroscopic properties in the estuarine system Ria de Aveiro

Luísa Santos, Newton C. Marcial Gomes, Eduarda B. H. Santos, João Miguel Dias, Ângela Cunha, Adelaide Almeida

University of Aveiro, Aveiro, Portugal

The influence of photochemical transformations of colored dissolved organic matter (CDOM) on microbial communities was evaluated in the estuarine system Ria de Aveiro. Two sites, representative of the marine and brackish water zones of the estuary were surveyed regularly in order to determine seasonal and vertical profiles of variation of CDOM properties. Optical parameters of CDOM indicative of aromaticity and molecular weight were used to establish CDOM sources and, microbial abundance and activity was characterized. Additionally, microcosm experiments were performed in order to simulate photochemical reaction of CDOM and to evaluate microbial responses to light-induced changes in CDOM composition. The CDOM of estuarine zones showed different spectral characteristics, with significant higher values of the specific ultra-violet absorbance at 254 nm (SUVA254) (5.5 times) and of the absorption coefficient at 350 nm (a350) (12 times) and lower ratio SR (S275-295/S350-400) at brackish water compared with the marine zone, reflecting the different amounts and prevailing sources of organic matter, as well as distinct riverine and oceanic influences. At the marine zone, the abundance of bacteria and the activity of Leu-AMPase correlated with a350 and a254, suggesting a microbial contribution to HMW CDOM pool. The irradiation of DOM resulted in a decrease of the values of a254 and a350 and in an increase of the slope S275-295, and of the ratios E2:E3 (a250/a365) and SR, which in turn increase its bioavailability. However, the extent of photoinduced transformations and microbial responses was dependent on the initial optical characteristics of CDOM. In Ria de Aveiro both photochemical and microbial processes yielded optical changes in CDOM and overall result of these combined processes determine the fate of CDOM in the estuarine system and have influence on local productivity and in adjacent coastal areas.

P051

Paracrine activation of Matrix Metalloproteinase (MMP)-2 and MMP-9 by skin cells exposed to UVB radiation

Andréa Costa Fruet, Franciele Knebel, Silvya Stuchi Maria-Engler, Silvia Berlanga de Moraes Barros

University of São Paulo, São Paulo, Brazil

Chronic skin exposure to UVB radiation leads to clinical changes characteristics of photo aged skin manifested as deep wrinkles, skin depigmentation, telangiectasia and is characterized by a complete perturbation of the dermis regarding extracellular matrix components and structure. These alterations have been associated with matrix metalloproteinases (MMP) increased activity. The mechanism involved in the induction of MMP by UV radiation may involve direct effects on the signaling cascade regulating MMP gene expression or indirect effects on inflammatory cytokines secretion that can modulate MMP expression and activity. The use of skin cells cultures to measure MMP induction and activity may be useful to the comprehension of the mechanisms of UV induced skin lesions and in the research for new photoprotective compounds. The aim of this study was to evaluate the paracrine activation of UVB irradiated skin keratinocytes (HaCaT) on MMP-2 and 9 secretion, activity and expression by normal human skin fibroblasts (NHSF).

HaCaT and NHSF cells were exposed to 0 – 32 mJ/cm^2 of UVB (TL 20W/12RS, Philips, Amsterdam, Holland). After 24 hours the HaCaT medium was removed and placed in a non-irradiated confluent NHSF culture 48 hours. MMP-2 and 9 activity and protein content was analyzed respectively by zymogram and western blot. IL-6 and TNF-α were evaluated by ELISA (R&D Systems, Minneapolis, MN, USA).

UVB radiation induces a decrease of proMMP-2 secretion by HaCaT from 10 mJ/cm^2 with no alteration in proMMP-9 activity. NHSF secreted only proMMP-2 with no alteration after UVB exposure. When the medium from UVB-irradiated HaCaT was placed in non-irradiated NHSF culture, there was an increase in proMMP-2 activity after 48 hours (24 and 32 mJ/cm^2 UVB; $p<0.05$ and $p<0.01$). Levels of IL-6 and TNF-α released by HaCaT were augmented in a dose-dependent manner after 24 hours of UVB treatment. Although some authors have demonstrated that IL-6 and TNF-α can induce MMP gene expression, no alteration on MMP-2 and 9-protein expression was observed in NHSF treated with HaCaT UVB-irradiated medium, with only higher activity of pro MMP-2.

Our results suggest that the activity of MMP-2 in the dermis promoted by exposure to UVB radiation in the skin involve paracrine activation of fibroblasts by cytokines, as TNF-α and IL-6, released by keratinocytes in a time and dose-dependent manner.

P052

Comparison of different methods for the assessment of skin aging parameters – an exploratory study in a group of 83 female patients

Markus Grabenhofer[1], Alois Schmalwieser[2], Harald Maier[1]

[1]*Department of Dermatology, Medical University of Vienna, Vienna, Austria;* [2]*Department of Biophysics and Statistics, Veterinary University of Vienna, Vienna, Austria*

At present assessment of the skin aging is mostly done by clinical inspection. This is investigator-dependent and therefore highly variable and poorly reproducible. There is urgent need for objective biometric data especially for the evaluation of the effectiveness of anti-aging products. The goal of our study was to establish a simple, however, highly reliable and reproducible method to determine the condition of the skin objectively.

We evaluated the reliability of different methods to measure skin aging parameters reported in the literature: elastometry, corneometry, sebumetry (Cutometer MPA580, Courage+Khazaka, Cologne, Germany), colorimetry (CR 300 Chromameter, Minolta, Marunouchi, Japan), and sonography (Esaote MyLab2, Esaote, Cologne, Germany) in a group of female test persons. Inclusion criteria were: skin type I-IV, age 20-60 years, BMI less than 28, no history of any cosmetic procedures in the past. Smokers or persons with a history of tobacco smoking were excluded. We defined 16 subgroups, one for each decade and skin type. The total of all test persons was distributed evenly among these subgroups. The biometric parameters were assessed in well-defined areas: temple (T), cheek (C), volar site of the upper arm (A), and buttock (B). Before measurement the test persons cleaned the skin with Octenisept® (Schülke&Mayr GmbH, Norderstedt, Germany) and ran through an acclimatization period of 30 minutes in an air-conditioned environment (T=24.8°C, relative humidity=39.6%). Elastometry was measured, both, as elastic deformation after 1 suction step and as stiffness by a repetitive course of 10 suction steps. Skin surface moisture and sebum is given as technical unit calibrated for cutometer MPA580. By using a 20MHz ultrasound transducer we measured the overall thickness of the cutis (mm) and the sub-epidermal low echogenic band (SLEB in mm). The colour of the skin in the test areas was measured in three different positions and is given as individual typology angle (ITA).

83 subjects participated in the study: 22 in the sub-group 20-29 (mean 23,3) years, 20 each in the sub-groups 30-39 (mean 34,6) and 40-49 (mean 42,6) years of age. The group between 50-60 (mean 54,4) years consisted of 21 women. 20 test persons were skin type I. The respective numbers for the sub-groups with skin type II, III, and IV were: 25, 22, and 16 test persons. Elasticity decreased with increasing age in all measured areas and for all skin photo types, significantly in area C and A. The difference between the skin types in the same age group, however, was not significant. Surprisingly, corneometry showed higher skin moisture values with increasing age, statistically significant in B. We found significantly higher moisture values in A for dark skin types compared to fair complexioned persons. No significant results could be found in the sebumetric measurements. We could not detect any significant change in the thickness of the cutis with age. The SLEB could only be detected in the UV-exposed areas (T, C) most significantly in patients with high cumulative UV exposure. There was no correlation of width of SLEB with age or skin photo type. The skin colour decreased significantly with age in C, with photo type in A and B. The decrease was significant in skin types III or IV. However, we could not find any significant change of colour in skin type I or II.

Our study shows that all methods to assess skin aging parameters except sebumetry produce valid results. Elastometry and sonography are the most promising techniques to assess skin aging parameters. The elastometric data describe the overall skin aging process whereas SLEB measured by ultrasound appears to be very specific for actinic skin damage.

P053

Aqueous extracts of cryophilic microalgae antagonize UVA induced disruption of gap junctional intercellular communication

Anna-Sophia Bothe[1], Felix Jorde[2], Thomas Leya[2], Rüdiger Greinert[3], Beate Volkmer[3], Dieter Pollet[1]

[1]*University of Applied Sciences Darmstadt, Darmstadt, Germany;* [2]*Fraunhofer IBMT, Pottsdam-Golm, Germany;* [3]*Elbekliniken Stade-Buxtehude, Dermatology Center, Buxtehude, Germany*

Gap junctional intercellular communication (GJIC) is an important mechanism to maintain tissue homeostasis and metabolic cooperation by intercellular exchange of low molecular weight molecules. It is well established that tumor-promoting agents such as phorbol 12-myristate 13-acetate (PMA) strongly inhibit GJIC resulting in a disruption of metabolic cooperation. More recently, it has been reported that also physical agents such as ionizing radiation, heat, and UV exposure differently affect GJIC (Provost 2003). On the other hand an enhancement or preservation of GJIC has been demonstrated for several dietary components exerting antioxidant or radical-scavenging efficacy (Siegler 1993).

The aim of this study was to investigate the effects of low-dose UV-A irradiation on GJIC in the human epidermal keratinocyte cell line HaCaT, in comparison to PMA treatment. Furthermore, the ability of β-carotene and a series of microalgal extracts, characterised by high antioxidant activity, to retain GJIC was assessed.

For this purpose the *Scrape Loading and Dye Transfer Technique* initially described 1987 by El-Fouly *et al.* was used. Confluent monolayers of HaCaT cultures were scraped in the presence of lucifer yellow (LY) as a gap junction permeable tracer, which becomes incorporated by cells along the scrape (3). GJIC activity was determined by counting LY^+ cells adjacent to the scratched cells.

Our results showed that UV-A exposure of HaCaT cells resulted in an immediate and dose-dependent reduction of GJIC. Irradiation with 24 J/cm^2 UV-A led to a 40% decrease in GJIC, whereas an one hour treatment with 10 ng/ml PMA inhibited GJIC down to 30%. Remarkably, neither PMA nor irradiation with UV-A considerably affected cellular viability, as assessed with the NRU assay. A 24 hour pre-treatment with 10 µM β-carotene or some, but not all, of the microalgal extracts significantly but not completely antagonized the PMA mediated inhibition of GJIC in HaCaT cells. Interestingly, we were able to show that also UV-A mediated downregulation of GJIC could be similarly attenuated by the same pre-treatment regimen.

Taken together, GJIC is a direct target of UV-A irradiation in human epidermis, even at low exposure-relevant doses. UV-A induced disruption of GJIC can be counteracted by chemopreventive agents sharing in common an antioxidant or radical scavenging efficacy. Thus, our findings may contribute to the development of novel strategies for radioprotection against UV-A mediated photo-ageing and tumorigenesis.

P054

Analyses of UV induced DNA mutations and repair

Katja Matt, Katharina Burger, Daniel Gebhard, Jörg Bergemann

Albstadt-Sigmaringen University of Applied Sciences, Sigmaringen, Germany

Human skin is constantly exposed to UV radiation which may cause severe DNA damages. The UVB part of the sunlight causes damages directly, as it interacts with the DNA bases resulting for example in cyclobutane pyrimidine dimers (CPDs) and pyrimidine-(6-4)-pyrimidone photoproducts (6-4PPs). Oxidative damages are caused by the UVA part of the sunlight in consequence of oxidative stress resulting in 8-oxoguanine (8-oxo-dG) for example. To avoid the formation of DNA mutations in consequence of these damages, the human cells provide efficient defence mechanisms like nucleotide excision repair (NER) and base excision repair (BER). NER is thereby responsible for repairing bulky DNA lesions like CPDs and 6-4PPs, whereas BER removes small, non-helix-distorting base lesions. In the context of BER the glycosylase OGG1 is of special interest as it recognizes and excises 8-oxo-dG, the most common oxidative DNA damage. Not only DNA damages but also UV-induced mutations for example CC to TT transitions are of special interest in context of human skin.

Here we present different quantitative methods for measuring DNA repair capacity (NER), incision activity of human OGG1 (BER) and a real-time PCR method to detect and quantify a CC to TT transition in mitochondrial DNA.

We developed a modified host cell reactivation assay (HCRA) for measuring nucleotide excision repair capacity in human skin cells. HCRA is a powerful tool to measure NER capacity as directly shows the functional recovery of a damaged plasmid using flow cytometry-based single cell analysis. In brief, cells are transfected with two reporter plasmids coding for green and red fluorescence respectively. One plasmid is damaged by UV radiation and serves as reporter for repair and the other plasmid serves as transfection control. If the cell is capable repair competent, both proteins are synthesized, if not only the undamaged plasmid is expressed. The amount of reporter protein synthesized from the previously damaged plasmid is directly proportional to the cells' repair capacity.

To measure the incision activity of hOGG1, we are establishing a cutting edge real-time assay using double stranded oligonucleotides containing 8-oxo-dG as substrate for hOGG1. This assay is accomplished on LightCycler 480 (Roche Applied Science) in a multi-well format. The strand which contains the 8-oxo-dGbase is labelled with a fluorescence dye, the other strand has a quencher attached. If hOGG1 excises 8-oxo-dG, the double stranded oligonucleotide gets instable and fluorophore and quencher are separated and a fluorescence signal can be detected. Therefore the repair kinetics of hOGG1 can be measured in real time.

The third method is a real time PCR assay to quantify a CC to TT transition in mitochondrial DNA at bases 591/592. CC to TT transitions are known as UV fingerprint mutations and may be the consequence of UV-induced CPDs in the worst case. As mitochondria do not possess NER, monitoring of a mitochondrial CC to TT transition is interesting concerning UV exposure of human skin and skin cells.

All three assay presented here contribute to an increase in knowledge about UV induced DNA damage, mutations and especially DNA repair.

P055

Visualization of vitamin D photosynthesis using Provitamin-D-doped nematic LC

Irina P. Terenetskaya, P.S. Kapinos, T.M. Orlova

Institute of Physics NAS Ukraine, Kiev, Ukraine

Initiation of endogenous synthesis of vitamin D in human skin is important biological function of solar UV-B (280-315 nm) radiation, and an *in situ* control of the vitamin D synthetic capacity of sunlight demands particular care in view of its dramatic seasonal and latitudinal changes.

The UV-B portion of sunlight converts provitamin D into previtamin D, which, in turn, undergoes thermoconversion into vitamin D at body temperature. Consequently, accumulation of previtamin D during UV exposure can serve as a measure of biological UV dose.

It has been shown that dissolution of optically active 7-Dehydrocholesterol (7-DHC, provitamin D3) molecules in liquid crystalline (LC) matrix provides the easiest detection of previtamin D synthesis [1,2] because upon UV-B irradiation the alteration of molecular geometry of provitamin D by the photoinduced conversion into previtamin D (with its further *cis-trans* isomerization into tachysterol) significantly affects the cholesteric pitch. For the first time, significant effect of UV irradiation on the number of Cano-Grandjean stripes in the wedge-shaped cell has been observed, that can be applied for personal UV dosimetry *in situ* [1]. Later on the shift of selective reflection peak and, as a result, the LC cell colour change, was observed under UV irradiation in a sandwich-like LC cell. This could provide the visual detection of previtamin D synthesis and evaluation of the accumulated UV dose *in situ* by comparison of the LC cell colour with the calibration scale [2].

Recently a new method for visual detection and measurement of the biologically active UV dose has been proposed [3]. It is based on the determination of an azimuth of a disclination line in a so-called θ-cell with unidirectional aligning of the LC director at one side and circular aligning at another one. Experimental observation of disclination rotation was performed with a composition of LC-805 (KANTO Chemical Japan) and 7-DHC (C = 0.65 wt.%, (Sigma). Unique correspondence was revealed between dynamic behavior of the disclinaton line and previtamin D accumulation *in vitro*.

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P056

Intercomparison of increase in serum 25-hydroxyvitamin-D3 in humans with previtamin D3 synthesis in vitro after sunbed exposures

Tetiana Orlova[1], Irina P. Terenetskaya[1], Asta Juzeniene[2], Johan Moan[2,3], Zoya Lagunova[2], Lage Aksnes[4]

[1]Institute of Physics NAS Ukraine, Kiev, Ukraine; [2]Institute for Cancer Research, Oslo University Hospital, Oslo, Norway; [3]Institute of Physics, University of Oslo, Oslo, Norway; [4]Department of Clinical Medicine, Section of Pediatrics, University of Bergen, Bergen, Norway

Ultraviolet (UV) radiation is liable to cause skin cancer but it is the main source of vitamin D. UV dosimetry is needed to obtain an optimal vitamin D status when skin cancer risk is minimal. A biological dosimeter of vitamin D synthetic UV radiation ('D-dosimeter') has been introduced earlier on the basis of an *in vitro* model of previtamin D photosynthesis [1].

For the first time *in vivo* generation of 25-hydroxyvitamin D (25(OH)D) in serum of healthy volunteers exposed to UV radiation from the sunbed (Wolff Suveren 53IG) was

accompanied by *in vitro* measurements of vitamin D formation using 'D-dosimeter'.
Statistical analysis for the three groups of volunteers (with different initial 25(OH)D level) before and after all sunbed exposures showed that the median value of 25(OH)D in serum increased in all three groups. It appeared that a rapid increase in serum 25(OH)D concentrations observed after the first sunbed sessions was slowed down with further exposures, and the values of 25(OH)D level obtained after 15 and 20 sessions were almost equal [2].
Such non-linear character of 25(OH)D formation *in vivo* is similar to previtamin D accumulation *in vitro.* Moreover, the linear correlations with high correlation coefficients R between *in vivo* changes of 25(OH)D distribution median in the three volunteer groups and *in vitro* accumulated concentration of previtamin D has been revealed, and the parameters of linear fit $C_{25(OH)D,\ nmol\ /\ L} = A + B * C_{PreD,\ \%}$ for three volunteer groups were determined.
In conclusion we note that linear correlations between *in vivo* and *in vitro* data could allow to use the single measurement of the initial serum 25(OH)D concentration in blood sample with further calculations of 25(OH)D level for different UV exposures for predictions of vitamin D status based on the measurements of previtamin D accumulation *in vitro*. Certainly, obtained in this study correlations should be re-measured for sunbed equipped with other fluorescent lamps because previtamin D photosynthesis strongly depends on the irradiation spectrum of an UV source. Furthermore these data need to be detailed depending on age, BMI, Fitzpatrick skin type, presence or absence of regular vitamin D intake, etc., that requires large epidemiological studies for completeness statistics.
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P057
A computational model for previtamin D3 production in skin

Merve Meinhardt-Wollweber[1], Ronald Krebs[2]
[1]Hannoversches Zentrum für Optische Technologien, Leibniz Universität Hannover, Hannover, Germany; [2]krebs - sciResolve - scientific research solutions, Hannover, Germany

Low levels of vitamin D have been implicated in a wide variety of health issues from calcemic diseases to cancer, diabetes and cardiovascular disease. For most humans, the majority of vitamin D_3 is derived from sunlight. How much vitamin D is produced under given exposure conditions is still widely discussed. We present a computational model for the production of (pre-vitamin D within the skin. It accounts for spectral irradiance, optical properties of the skin and concentration profile of provitamin D.
Taking the photosynthesis of previtamin D as a simple photochemical process, the amount of previtamin D formed depends on effective spectral irradiance and available educt provitamin D within the skin. The UV component of the spectral irradiance is strongly attenuated within the skin. On its way through the first hundred microns of the skin, it is diminished by about two orders of magnitude in the UVB wavelength range. Given this strong dependence of available radiation on depth within the skin, the total amount and distribution of the educt provitamin D within this small range is crucial for the total amount of previtamin D_3 produced. To compute the photoinduced production of vitamin D in skin, the spatial distribution of the irradiating light within the skin was calculated. The spectral irradiance as attenuated by the optical properties of the skin is calculated for the upper 200 µm of human skin (other depth ranges may be chosen). Only absorption is considered for the attenuation of UV radiation as scattered photons are still available for the photoisomerization reaction. The light distribution within the skin is then weighted with the chosen response function and with the concentration profile of the educt provitamin D to calculate the total amount of previtamin D produced for a given set of parameters. As response function, an action spectrum from an in vitro model, 7-DHC in ethanol, was chosen.
We show results computed for various sets of parameters yielding the distribution of produced previtamin D in the skin.

P058
Study of tetrahydrobiopterin photochemistry: *in vitro* and *in silico*

Andrey A. Buglak, Taisiya A. Telegina, Tamara A. Lyudnikova, Yuliya L. Vechtomova, Mikhail S. Kritsky
A.N. Bach Institute of Biochemistry of RAS, Moscow, Russian Federation

Pterins absorb ultraviolet and are the targets for UV radiation action on the human organism. Tetrahydrobiopterin (BH_4) serves as a cofactor of aromatic amino acid hydroxylases (AAHs), NO synthases and alkylglycerol-monooxygenase. Products of BH_4 oxidation accumulate in depigmented patches of skin of vitiligo patients. Vitiligo is associated with disturbance of melanin biosynthesis, the first stage of which is the BH_4-dependent hydroxylation of phenylalanine. It appears that in vitiligo tetrahydrobiopterin regeneration cycle is disrupted, and therefore its oxidized forms accumulate in depigmented cells. These oxidized pterins are able to photosensitize molecular oxygen and, thus, cause oxidative stress. Thereby, we studied photooxidation of BH_4.
We irradiated BH_4 solutions (pH 7,2) with a wide range UV (290-400 nm) or with monocromatic UV (λ 300 nm and 350 nm). Identification of products was based on HPLC separation, using photometric, fluorometric and amperometric detection of eluted compounds in combination with the analysis of their absorption and fluorescence spectra.
We have shown that broadband UV-irradiation accelerates BH_4 oxidation in presence of O_2. However, monochromatic UV irradiation at BH_4's maximum (λ_{max} 298 nm) had a low effect on its oxidation rate and had a quantum yield (Φ_{300}) 7,7±0,7%. On the other hand, BH_4 oxidation under 350 nm irradiation had Φ_{350}= 30,7±3,0%. Apparently, oxidation of BH_4 is sensitized by oxidized pterins. And in this system the most active sensitizer is biopterin (Bp), which is a product of BH_4 autoxidation. We have shown that the rate of BH_4 oxidation ($V_{ox\text{-}BH_4}$) under UV-irradiation depends on the concentration of Bp in solution and linearly depends on the UV intensity.
There are two possible mechanisms of biopterin impact: I – through formation of reactive oxygen species, and II – direct interaction of excited Bp with BH_4. Using Density Functional Theory (DFT) method we have shown that first mechanism is realizable through formation of singlet oxygen $^1O_2$ by excited triplet biopterin ^3Bp (eq. 1) and, obviously, results in formation of dihydrobiopterin (BH_2) and H_2O_2 (mechanism Ia). While the mechanism which starts with electron transfer from ^3Bp to O_2 (eq. 2) and develops with reaction between $O_2^{\cdot -}$ and BH_4 (mechanism Ib) looks improbable. Another mechanism (mechanism II) that starts with the direct reaction between ^3Bp and BH_4 (eq. 3) is also likely. Emerged free radical species $Bp^{\cdot -}$ and $BH_4^{\cdot +}$ may further participate in BH_4 oxidation, in particular, through activation of molecular oxygen and production of $O_2^{\cdot -}$.

$$^3Bp + O_2 \rightarrow Bp + {}^1O_2 \quad (1)$$
$$^3Bp + O_2 \rightarrow Bp^{\cdot +} + O_2^{\cdot -} \quad (2)$$
$$^3Bp + BH_4 \rightarrow Bp^{\cdot -} + BH_4^{\cdot +} \quad (3)$$

Based on calculations of Gibbs free energy of reactions between BH_4 derivatives and reactive oxygen species, as well as reactions of photosensitization, we proposed a detailed mechanism of BH_4 photooxidation.
Further experiments confirmed our preliminary DFT calculations. Addition of 250 µM of KI, which is a quencher of triplet pterins, to reaction mixture (43 µM BH_4 and 40 µM Bp) reduced the rate of BH_4 oxidation ($V_{ox\text{-}BH4}$) by 57,3±0,5% under

λ 350 nm irradiation. That means ^{3}Bp participation in the process of BH_4 photooxidation is very likely. Under the same conditions in D_2O (lifetime of $^1O_2$ in D_2O increases up to 22-fold) $V_{ox\text{-}BH4}$ increased by 92,4±1,6%, that means direct energy transfer from ^{3}Bp to O_2 happens and mechanism Ia is realized. The addition of superoxide-dismutase (SOD) under λ 350 nm irradiation reduced $V_{ox\text{-}BH4}$ by 6,2±3,6%, which shows a low contribution (or even absence) of mechanisms Ib and II. Same effects of KI, D2O and SOD on BH_4 oxidation with λ 300 nm irradiation led us to the idea that in this case oxidation also goes through sensitization by oxidized pterins. Oxidized pterins are always present in solution as impurities of initial BH_4 and as products of its autoxidation, and they absorb UV with λ 300 nm. Thereby, we suppose that BH_4 itself is photochemically inactive.

It appears that similar mechanisms are involved in the development of pathological processes in vitiligo. Sensitization by oxidized pterins leads to production of reactive oxygen species and to oxidative stress. Oxidative stress suppresses work of enzymes such as pterin-4a-carbinolamine dehydratase (PCD). Inhibition of PCD disrupts regeneration cycle of BH_4 what leads to failure of normal functioning of BH_4-dependent AAHs and suppression of melanogenesis. We presume that in depigmented skin cells BH_4 is oxidized producing more oxidized pterins, which leads to further progression of vitiligo pathology.

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P059

Peptide-targeted photosensitisers via efficient 'click' conjugations in solution

Ruggero Dondi[1], Elnaz Yaghini[2], Marilena Loizidou[2], Alexander J. MacRobert[2], Ian Michael Eggleston[1]

[1]*Department of Pharmacy & Pharmacology, University of Bath, Bath, United Kingdom;* [2]*UCL Medical School, University College London, London, United Kingdom*

The conjugation of photosensitisers to proteins or targeting peptides is an attractive way of obtaining chromophores with improved aqueous solubility and tissue selectivity for applications in both PDT and light-activated drug delivery. A versatile synthetic approach for the synthesis of such derivatives involves the bioorthogonal ligation of a suitably functionalized photosensitizer with a completely unprotected peptide in solution [1]. This avoids potentially complicated final deprotection strategies that may be required in protocols involving couplings of photosensitisers to peptides on solid phase.

In this communication, we will present the preparation and preliminary characterisation of a variety of porphyrin and chlorin derivatives, generated via azide-alkyne "click" chemistry with a suitably functionalised peptide component. In particular, we explore the ligation of clinically important photosensitisers to cationic cell-penetrating peptides (e.g. HIV Tat 48-57) and peptides that are designed to target the epidermal growth factor receptor (EGFR), which is overexpressed in a range of cancer types.

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P060

Photoinduced spectral changes of MES-capped gold nanoparticles

Marija Matulionyte[1,2], Raminta Marcinonyte[2], Ricardas Rotomskis[1,2]

[1]*Biomedical Physics Laboratory, Institute of Oncology, Vilnius University, Vilnius, Lithuania;* [2]*Biophotonics Group of Laser Research Centre, Vilnius University, Vilnius, Lithuania*

Gold nanoclusters (AuNCs), composed of several to tens of atoms, have recently attracted increased attention due to their unique molecule-like properties, such as discrete electronic states and size dependent photoluminescence wavelength. More importantly, they exhibit several advantageous properties over the semiconductor QDs in biomedical applications including excellent photostability, low toxicity, ultimately small sizes and facile synthesis. However, photoluminescence stability under irradiation with UV/blue light used for excitation is usually unknown even though it is one of the most important properties to be investigated before applying photoluminescent AuNCs for imaging of biological objects.

In this work we present photoluminescence stability of 2-(N-Morpholino) ethanesulfonic acid (MES)-stabilized gold nanoparticles (Au-MES NPs) under irradiation with UV/blue light. Synthesized Au-MES NPs exhibited blue PL at 476 nm wavelength. Absorption spectrum did not coincide with PL excitation spectrum (λ_{max}=420 nm) showing that there are bigger than 2 nm Au-MES NPs in synthesized solution.

Irradiating Au-MES NPs with 402 nm wavelength light, PL intensity first decreased by 44 % and PL band shifted to shorter wavelength region by 47 nm. PL intensity increased by 14.5 % as irradiation dose of 21.8 W*s/cm^2 was accumulated. More photostable photoluminescent Au-MES NPs were formed and further irradiation led to slow decrease of PL intensity. Irradiating the sample with 330 nm wavelength light PL intensity of Au-MES NPs increased while absorbance at 330 nm wavelength decreased. Non-luminescent nanoparticles, that quenched PL of the sample, were disrupted.

P061

Photoclavage of Phosphodiester bonds in DNA or RNA by copper complexes

Mohammed Oudeh Ismael

Master Degree in Clinical Laboratory Science, Birzeit University, Ramallah, Palestinian Territory, Occupied

Nucleic acids are made up from their forming units, namely nucleotides via the linkage of 3,5-phosphodiester bonds, Under the pseudo-physiologic condition, the phosphodiester bond in DNA is extremely stable. At pH 7, its half-life is as long as several hundreds of billions years, much longer than the age of the earth itself, to hydrolyze the phosphodiester bond in DNA within a few minutes, a catalyst would have to provide over 10^{17} fold rate acceleration. the great stability of phosphodiester bond is thought to be one of the reasons why the nature chooses DNA as the genetic material.

Cleavage of DNA is an essential process in all living systems. For example, topoisomerase enzymes resolve topological problems of DNA in replication, transcription and other cellular transactions by cleaving one or both strands of the DNA. Another example are restriction enzymes (or restriction endonucleases), which protect the cell against virus infection by cleavage of the foreign DNA, or by degrading cellular DNA during apoptosis (programmed cell suicide) of the affected cell. Finally, the activity of many anticancer drugs rely on their ability to introduce extended damage to the DNA in the (affected) cells (*e.g.* bleomycin), which can trigger apoptosis, leading to the cell death.

In general, three different types of DNA cleavage can be distinguished, namely i) DNA hydrolysis, ii) photochemical cleavage, and iii) oxidative cleavage, although the last two categories are quite closely related.

The hydrolytic cleavage mode is different from the oxidative mode it only cleaves the phosphodiester bonds in the nucleic acid strands in a hydrolytic path, usually the cleavage products are sticky and connectable, hydrolytic cleavage eliminates those cytotoxic side effects due to the emergence of ROC and the resulting fragments are easy to relegated the hydrolytic reaction of DNA can be simplified as a two steps mechanism, firstly a nucleophile produced via metal activated water molecule attacks the phosphorus atom, forming a penta-coordinated intermediate, and this step is reversible, secondly, the 2-deoxyribonucleotide fragments with a 3'-OH group are removed from the phosphorus through the fission of the P-O bond, and the product is formed.

Photochemical cleavage of DNA can be subdivided in two categories: i) direct UV-induced DNA damage and ii) DNA cleavage via the generation of reactive (oxygen) species, by photochemical means. The former involves a direct UV-promoted dimerization reaction between two pyrimidine residues (*i.e.* thymine and cytosine) to form mutagenic and cytotoxic DNA lesions.

In both photochemical and oxidative DNA cleavage often similar reduced oxygen species are generated as reactive intermediates. In photochemical DNA cleavage these intermediates are generated by organic compounds or metal complexes (Scheme 2) It is important to note here that most DNA-cleaving agents are not directly responsible for the DNA damage observed, but they generate highly reactive intermediates, which cause DNA damage (*e.g.* sugar or nucleobase modification). In turn, the damaged DNA can undergo auto-oxidation reactions leading to strand scission. Alternatively, labile sites are generated in the DNA, which require further treatment (such as alkaline workup) to effect strand scission.

DNA damage initiated by photosensitization can be divided in two major types; a one electron process (Type I process), and a pathway involving singlet oxygen (Type II process). In the first type (Type I process), the cleaving agent is excited and generates sequentially a superoxide radical from molecular oxygen via an electron transfer step. Superoxide itself is a rather poor oxidant, and it can be further reduced (leading to H_2O_2 and ·OH) or it can function as a reductant. The DNA damage observed via this pathway is mainly guanine oxidation, formed via guanine radical cations. This results in the formation of base labile sites in the DNA.

In a Type II process, the photo excited compound generates singlet oxygen, which only modifies guanine residues, in contrast to superoxide. Two pathways can be distinguished. A Diels-Alder reaction with singlet oxygen results in the formation 4,8-dihydro-4-hydroxy-8-oxo-dG, and after further reduction in 8-oxo-dG. A [2+2] cycloaddition with singlet oxygen results after a cascade of reactions in the formation of cyanuric acid. The modified residues are base labile positions in the DNA and alkaline workup is required to initiate strand breaks.

Oxidative cleavage often requires the addition of exogenous agents such as hydrogen peroxide or irradiation of light with a certain frequency, thus initiating the formation of active species such as atomic oxygen or hydroxyl free radicals which are real initiator of the reaction via an oxidative mechanism. Enediyne antibiotics and their analogues as iron-bleomycin and other metal complex have been applied to oxidative cleavage of DNA.

Oxidative cleavage usually needs co-reactant to produce reactive oxygen species (ROS) which is the real initiator for the reaction. However ROS has evident cytotoxic effects and the resulting DNA fragments from oxidative cleavage are not connectable. In oxidative mechanism the system initiate the cleavage of nucleic acid by forming free radicals which have the ability to give rise to dehydrogen oxidation of the ribose rings and phosphate backbone on the nucleic acids, and then lead to the cleavage of phosphodiester bonds.

P062

Influence of polyethyleneglycol side chains on oxygen and drug permeability of PLGA nanoparticles

Ester Boix[1], Santi Nonell[1], Maria Lluïsa Sagristà[2], Margarita Mora[2]

[1]*Institut Químic de Sarrià-Universitat Ramon LLull, Barcelona, Spain;* [2]*Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Barcelona, Barcelona, Spain*

Biodegradable poly(D,L lactide-co-glycolide) (PLGA) nanoparticles are being extensively explored as drug delivery systems for chemotherapy or photodynamic therapy (PDT) [1, 2]. In this study, a model porphyrin (ZnTPP) was encapsulated in non-coated and 10%PEG-coated PLGA nanoparticles, and the influence of PEG decoration was evaluated in their physico-chemical and photobiological properties. PEGylation afforded smaller nanoparticles with lower zeta potential, as previously observed [3]. Entrapment efficiency was, however, similar for both systems, and absorption and emission spectroscopy showed that ZnTPP is found mainly in monomeric form in them. The kinetics of singlet oxygen luminescence reveals that the presence of the more hydrophilic polymer PEG makes a fraction of ^{3}ZnTPP more accessible to oxygen. More interestingly, the evaluation of singlet oxygen formation kinetics as a function of temperature demonstrate the thermosensitive properties of these polymers, showing that as temperature increases nanoparticle matrix becomes more fluid, which is translated into a shorter triplet and singlet oxygen lifetimes. Moreover, sodium azide, which is not capable of deactivating singlet oxygen at room temperature in non-coated PLGA, exerts an extended effect at temperature higher than the glass transition temperature. Additionally, sodium azide is more effective for the PEGylated system, confirming that PEG decoration makes the nanoparticles more permeable. Biological studies indicate that although non-coated PLGA nanoparticles are internalized in a greater extent in HeLa cells, phototoxicity effects are similar for both sorts of systems, proving the major permeability of PEGylated nanoparticles.

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P063

Synthesis, Photochemistry, and Photocytotoxicity of Iodinated Chlorins

Toru Oba[1], Satoru Yasuda[1], Kyoko Fujiwara[2], Hiroaki Horiuchi[3], Satoshi Ito[1], Yoshinao Shinozaki[4], Joe Otsuki[4], Hiroki Nagase[5]

[1]*Department of Material and Environmental Chemistry, Utsunomiya University, Utsunomiya, Tochigi, Japan;* [2]*Nihon University School of Medicine, Itabashi, Tokyo, Japan;* [3]*School of Science and Technology, Gunma University, Kiryu, Gunma, Japan;* [4]*College of Science and Technology, Nihon University, Chiyoda-ku, Tokyo, Japan;* [5]*Chiba Cancer Center Research Institute, Chiba, Chiba, Japan*

Efficient generation of singlet oxygen is a requisite for PDT photosensitizers. Singlet oxygen is produced by excitation energy transfer from triplet excited state photosensitizer to ground state oxygen, and the triplet yield depends on intersystem crossing of the photosensitizer. It is expected that introduction of a heavy atom to a photosensitizing drug improve efficiency of singlet oxygen generation and efficacy of PDT.

Chlorins are promising PDT photosensitizers, iodine has long been used safely in clinical purpose. However, there has been no report on photodynamic properties of iodochlorins. Here in this presentation, we will report synthesis, photochemistry, and photocytotoxicity of iodochlorins. We revealed for the first time that heavy atom effect and photocytotoxicity of iodochlorins can be controlled by the position of the iodination.

We synthesized HPPH (1) analogs that possess an iodine atom at the C20-position (directly introduced to the p-system) or the C3^2-position (introduced to the peripheral substituents) by using I_2 and [bis(trifluoroacetoxy)iodo]benzene. The C20-iodinated-HPPH (2) did not show fluorescence, while the C3^2-iodinated-HPPH (3) gave weaker fluorescence than 1. Fluorescence quantum yields (in MeOH) were 0.13 (1), 0.00 (2), and 0.01 (3), and singlet oxygen yields (in MeOH) were 0.40 (1), 0.22 (2), and

0.53 (3). These values are consistent with their photocytotoxicity to T24 human bladder cancer cells. These compounds did not show any dark toxicity, and their photosensitizing activities showed dose-dependency. Compound 2 was less effective in killing cells than 1, while photocytotoxicity of 3 was the greatest among the three. Our study shows that external heavy atom effect can be a possible strategy to develop novel photosensitizing drugs.

P064

Biosynthesis of metals nanoparticles from plants

Shimaa M. Alexeree

National Institute of Laser Enhanced Science (NILES), Cairo University, Giza, Egypt

In recent years, the synthesis of nanoparticles is an expanding research area due to the potential applications for the development of novel technologies. Generally, nanoparticles are prepared by many techniques such as chemicals reduction of silver ions in aqueous solution by stabilizing agents, chemical reduction and thermal decomposition in organic solvent. These methods involve the use of toxic, hazardous chemicals, which may cause potential environmental and biological risks. So the development of green synthesis of nanoparticles is of great importance in the field of nanotechnology. The aim of the present investigation is to develop rapid and simple biosynthesis methods of silver nanoparticles (Ag NPs) in one step using Garlic (*Allium sativum*) and Potatoes plants. The results showed that gradual change in the color of plant extract from colorless to reddish brown after adding of silver nitrate indicates the silver nanoparticles (Ag NPs) synthesis. The characterizations of (Ag NPs) were determined by using (UV-VIS) spectrophotometry and transmission electron microscopy (TEM).

P065

Synthesis and photophysical studies of new chlorin e6 trimethyl ester derivatives

José C. J. M. D. S. Menezes[1], Maria A. F. Faustino[1], Kleber T. Oliveira[2], Artur M. S. Silva[1], Vitor F. Ferreira[3], Maria Graça P. M. S. Neves[1], Beate Roeder[4], José A. S. Cavaleiro[1]

[1]*Department of Chemistry & QOPNA, University of Aveiro, Aveiro, Portugal;* [2]*Department of Chemistry, University Federal of São Carlos, São Carlos SP, Brazil;* [3]*Department of Organic Chemistry, University Federal Fluminense, Niterói, Rio de Janeiro, Brazil;* [4]*Institut für Physik, Humboldt-Universität zu Berlin, Berlin, Germany*

The development of new drug molecules comprising natural porphyrin like chlorin-e_6 trimethyl ester and other pharmacologically relevant natural compounds like coumarin, quinoline and naphthoquinone derivatives should exhibit improved biological activities due to a synergic effect involving the two structural moieties. With this aim, herein we present the structural features of new chlorin e_6 derivatives synthesized *via* the recently developed domino Knoevenagel hetero-Diels-Alder reaction on porphyrins [1]. These hybrid molecules should have the advantage of being less toxic to normal tissue, since the basic core structure is derived from a natural chlorin e_6. The detail photophysical properties of these molecules will be discussed considering possible photodynamic applications, mainly photodynamic therapy (PDT) and photoinactivation of microorganisms (PDI).

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P066

Metal nanoclusters for biological applications

Oriol Planas, Santi Nonell

Institut Químic de Sarrià, Universitat Ramon Llull, Barcelona, Spain

In contrast to metal nanoparticles and bulky metals, metal nanoclusters are endowed with molecule-like behaviour showing a fascinating and versatile photoluminescence which can be tuned modifying their surface capping ligands [1]. Moreover, metal nanoclusters present good photostability and its photoluminescence lifetimes range up to the microsecond order [2]. As a result, they have been purposed as a promising new generation of fluorescent probes for time-resolved fluorescence microscopy.

Herein the photoluminescence of several gold and silver nanoclusters has been assessed including fluorescence lifetimes studies. Some of the nanoclusters analyzed showed long lived photoluminescence components which might interact with molecular oxygen through Dexter energy transfer giving rise to singlet oxygen as well as, other reactive oxygen species (ROS). Our results show that oxygen quenches (both reversibly and irreversibly) the nanocluster luminescence mostly by a static mechanism but no singlet oxygen seems to be produced as a result of such quenching.

In addition, some of the metal nanoclusters have been conjugated using different approaches to photosensitizers in order to explore their interaction and to evaluate the cluster effect on the production of singlet oxygen.

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P067

^{18}O-Labeled singlet molecular oxygen reaction with 1,N2-etheno-2'-deoxyguanosine

Marisa H.G. Medeiros[1], Glaucia R. Martinez[2], Paolo Di Mascio[1]

[1]*Universidade de São Paulo, Instituto de Química, Departamento de Bioquímica, São Paulo, Brazil;* [2]*Universidade Federal do Paraná, Departamento de Bioquímica e Biologia Molecular, Curitiba, Brazil*

Exocyclic DNA adducts are emerging as potential new tools for the study of oxidative stress-related diseases as well as the determination of cancer etiology and cancer risk. It is important to determine whether levels of exocyclic DNA adducts reflect redox stress in vivo and what role these adducts play in human diseases. To answer these important questions, inter-individual differences, tissue distribution, background levels, and repair has been assessed. An important aspect, which should be considered, is the chemical reactivity toward oxidants species generated during the oxidative stress process. To investigate the effect of singlet molecular oxygen on biomolecules such as DNA, we have devoted efforts to develop suitable singlet molecular oxygen generators. The combined use of the thermolysis of a water-soluble naphthalene endoperoxide as a generator of ^{18}O-labeled singlet molecular oxygen and the sensitivity of HPLC coupled to tandem mass spectrometry, allowed the study of singlet molecular oxygen reactivity toward etheno adducts. We report here studies related to oxidation of 1,N^2-etheno2'-deoxyguanosine (ethenodGuo) by ^{18}O-labeled singlet molecular oxygen. Products detection and characterization were achieved

using high performance liquid chromatography coupled to UV and electrospray ionization tandem mass spectrometry. A mechanistic pattern of singlet dioxygen-mediated oxidation of ethenodGuo leading to 2'-deoxyguanosine (dGuo) formation is proposed using isotopic labeling experiments coupled to mass spectrometry measurements. We found that dGuo is regenerated via reaction of singlet molecular oxygen with the etheno-linkage, having a dioxetane as an intermediate, which cleaves and loses the aldehyde groups as formate residues. In order to better understand this interaction, we determined the rate constant for singlet molecular oxygen quenching ethenodGuo and other etheno modified nucleosides. The rate constant (kt) values obtained for ethenonucleosides are comparable to kt of dGuo. For comparative purposes, the stability of the ethenodGuo was also investigated in solutions under Fenton-type reaction. The formation of dGuo was also observed. In conclusion, the reaction with singlet molecular oxygen and other radicals species should be considered when etheno-adducts are used as biomarkers of DNA damage.

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P068

The Bunsen-Roscoe reciprocity law is not fulfilled in apoptosis of lymphocytes induced by psoralen photooxidation products

Alla Kyagova, Pyatnitskiy Iliya, Malakhov Michail, Alexander Potapenko

Russian National Research Medical University, Moscow, Russian Federation

Psoralens are substances of plant or synthetic origin, which in combination with UVA light (PUVA/ photopheresis) are used in the treatment of certain T-cell-mediated disorders, such as psoriasis, vitiligo and cutaneous T-cell lymphoma. It is assumed that in the course of UVA-photolysis the psoralen photooxidation products (POP) are generated, which could contribute to immunosuppressive effects induced by PUVA/photopheresis. Previously we have demonstrated that POP possessed multiple biological activities, namely, they could increase erythrocyte membrane permeability (POP-hemolysis, 1988), induced the respiratory burst of phagocytes (1991), and modulated (suppressed/activated) contact (2001) and delayed (1996) hypersensitivity reactions. The latter gives reason to expect that POP-based drugs could be developed for the treatment of T cell-mediated skin diseases.

It is known that induction of apoptosis of immune cells may underlie the therapeutic effects of photopheresis. Caffieri et al. (2007) disclosed and characterized some POP products with pro-apoptotic properties. One of these products was 6-formyl-7-hydroxycoumarin (FHC), which in concentration of 50 μM resulted in the death of 50%[*]-Jurkat cells by apoptosis *in vitro*. Interestingly, the FHC had no any apoptotic effect on T lymphocytes isolated from fresh blood of healthy volunteers. However, it is clear that the generation of the FHC in so high concentration is not feasible in the real conditions of the treatment by PUVA/photopheresis.

The aims of our study were: a) to determine whether POP-products could induce apoptosis of lymphocytes in the range of concentrations that are different from used by Caffieri et al (2007) b) to study the feasibility of the Bunsen-Roscoe reciprocity law in the generation of pro-apoptotic POP-products; c) to evaluate how the aggregation of the POP-products can influence their pro-apoptotic properties.

By employing the annexin V and propidium iodide staining for apoptosis detection we have shown that crude mixture of POP-products induces 30% apoptosis in suspensions of both naïve and derived from the dinitrofluorobenzene-(DNFB)-sensitized-mice lymphocytes. Moreover, it was revealed that the total concentration of crude mixture of pro-apoptotic POP-products was not greater than 3 μM in cell suspensions. In this regard, we assume that the FHC may not be a product of the photo-oxidation of psoralen that is responsible for the induction of apoptosis in lymphocytes from naive or DNFB-sensitized mice. Comparable levels of apoptotic lymphocytes obtained from both naive and DNFB-sensitized mice indicate a minor role of apoptosis as the mechanism of the CHS-suppression induced by the POP-products.

It is known that immunsuppressive POP-products are better formed at low UVA-fluence rates, whereas the membranotoxic POP-products are better generated at high UVA-fluence rates. We have found that exposure of psoralen solution to UVA-light at high fluence rate (190 W/m^2, HI-UV) lead to increase in the apoptotic effect of the POP-products compared to POP-products, which were obtained at low fluence rate (40 W/m^2, LI-UV): at 9 kJ/m^2 fluence, the HI-UV-POP- and LI-UV-POP-products induced cell death of 32% and 7% of lymphocytes, compared to the control, correspondently. Moreover, LI-UV-exposure of psoralen solution in a fluence range from 6 to 15 kJ/m^2 leads to monotonous increase in the apoptotic effect of the POP-products. In contrast, the maximal apoptotic effect of the POP-products was observed at 9 kJ/m^2 fluence and then it decreased with the increase in the fluence if psoralen solution was irradiated by the HI-UV-light. Thus, the Bunsen-Roscoe reciprocity law was not fulfilled in apoptosis of lymphocytes induced by POP-products.

Using the resonance light scattering method we have shown that aggregation of POP-products occurs during the UVA-irradiation of psoralen solution. These aggregates were formed more effectively at HI-UV-light exposure rather than at LI-UV. These data indicate on a possible role of aggregation processes of the POP-products in the manifestation of their pro-apoptotic properties. Additionally we may propose two mechanisms of apoptosis induction by psoralen photoproducts, namely, with or without the participation of aggregates of psoralen photoproducts at low and at high fluence rates of UVA-light exposure, respectively.

P069

Aggregation of psoralen photolysis products monitored by the resonance light scattering: hypothesis of mechanochemical mechanism of hemolysis

Alexander Potapenko, Evgenii Nevezhin, Natalya Vlasova, Iliya Pyatnitskiy, Alla Kyagova, Michail Malakhov

Russian National Research Medical University, Moscow, Russian Federation

The Resonance Light Scattering (RLS) effect is observed as increased Rayleigh light scattering intensity at or very near the wavelength of absorption of an aggregated molecular species. RLS allows revealing extended supramolecular tightly packed aggregates of dyes when excitonic interaction exists between molecules of dye. RLS intensity (*I*) is proportional to squared polarizability (size) of aggregate: $I \approx |\alpha|^2$. The RLS spectrum allows for selective observation of aggregates, even in multicomponent systems that include a large fraction of monomers or other aggregates. [R. F. Pasternack, and P. J. Collings, *Science*, 269 (1995), 935-9].

By using the RLS technique we found that photolysis products of psoralen form aggregates under UVA irradiation (365 nm). The RLS spectra were registered by synchronous scanning of both monochromators of spectrofluorimeter preset to the same wavelength. The registered RLS spectra were corrected for sensitivity of spectrofluorimeter and inner filtering effects of the samples. The photoinduced aggregation was observed both in the presence and absence of molecular oxygen, but the shape of RLS spectra and the dose dependences were different. Photoinduced aggregates formed in the presence of oxygen and containing products of psoralen photooxidation (POP-aggregates) had two RLS maxima near 300 nm and 350 nm, and very broad shoulder extending to blue-green region.

The Bunsen-Roscoe law of reciprocity of intensity and time of irradiation is not fulfilled for the formation of photoinduced aggregates of psoralen: the amplitude of RLS signal drastically increased at increasing intensity of irradiation from 40 W/m^2 to 800 W/m^2 at constant dose, which indicates participation of secondary photochemical reactions in aggregate formation.
POP-aggregates are not stable and decompose on storage with decay half-life time varying from 0.5 hour for small aggregates to several hours for big ones.
The main patterns of formation and decomposition of POP-aggregates correlate well with those of psoralen photooxidation products, capable to induce hemolysis of erythrocytes. This correlation indicates the possibility of participation of POP-aggregates in distortion of biological membranes permeability, in particular the permeability of erythrocyte membranes.

P070

Furan-naphthoxazole dyads for singlet oxygen detection

Rubén Ruiz

Universitat Ramon Llull, Barcelona, Spain

Singlet oxygen ($^1O_2$) is well-known among reactive oxygen species due to its importance as a synthetic reagent and its involvement in pathological and physiological processes. Over the years, insight has been gained in the rules that obey its generation, the characters involved and the energy requirements. Despite the major progress made, a better understanding of $^1O_2$ behaviour in biological systems is still needed. Of critical importance, techniques and/or methods able not only to detect but also to quantify the concentration of $^1O_2$ both in solution and *in vivo* are still needed. $^1O_2$ can be detected through its intrinsic phosphorescence with maximum centered at 1275 nm. Trapping $^1O_2$ with suitable chemical acceptors is also extensively used. Fluorogenic probes that develop a bright fluorescence upon reaction with $^1O_2$ have attracted much interest lately as they offer excellent sensitivity and convenience.
A common drawback for all the fluorogenic probes developed up to date is that the fluorescence increases only moderately after reaction with $^1O_2$. Moreover, since electron-transfer reactions are strongly dependent on solvent polarity, false positive signals arise that merely reflect location of the probe rather than reaction with $^1O_2$. In addition, anthracene is frequently used as trapping moiety; but it is potentially misleading since anthracene itself may auto-oxidize the probe.
In this study we report the photochemical behaviour of a new family of naphthoxazole-based dyads capable of monitoring $^1O_2$ in solution with unprecedented sensitivity. The candidate dyads are composed by a furan as $^1O_2$ trap plus a naphthoxazole moiety linked directly or through an unsaturated bond to the oxazole ring. In the native state, the inherent great fluorescence of the naphthoxazole moiety is quenched; but in the presence of $^1O_2$ their fluorescence is boosted up to a factor of *ca.* 300-fold, at the optimal selected wavelength. Thus, the presented dyads outperform the commonly used indirect fluorescent singlet oxygen probes in terms of fluorescence enhancement. Its added selectivity towards $^1O_2$ and the negligible effects of self-sensitization, make naphthoxazole dyads worth of further scrutiny and development as $^1O_2$ fluorescent probes.

P071

Investigations of singlet oxygen generation by CdSe/ZnS - Chlorin e6 complex

Gediminas Spogis[1], Vilius Poderys[1], Ricardas Rotomskis[1,2]

[1]*Biomedical Physics Laboratory of Institute of Oncology, Vilnius University, Vilnius, Lithuania;* [2]*Biophotonics Group of Laser Research Centre, Vilnius University, Vilnius, Lithuania*

Cancer remains one of the leading causes of death in the world. There is constant interest in creating new treatment methods and developing existing ones, such as photodynamic therapy of cancer (PDT). PDT is based on combining a light-sensitive drug, called photosensitizer (PS), and light of appropriate wavelength to kill cancer cells by producing active oxygen species, including highly reactive singlet oxygen ($^1O_2$). There has been an extensive interest in quantum dots (QDs) as a promising material for improving PDT. QDs are semiconductor nanoparticles, known for their unique photophysical properties, such as narrow size-dependent photoluminescence (PL) spectra, high PL quantum yield, broad absorption spectra, large extinction coefficient and two-photon absorption cross section, high photostability and ability to functionalize QD surface. It has been shown that QDs can form complexes with conventional PSs and be used as light-absorbing energy donors for PSs, thus increasing the efficiency of PDT.
In this work we studied singlet oxygen generation of carboxyl functionalized CdSe/ZnS QDs and PS chlorin e_6 (Ce_6) complex. The formation of $^1O_2$ was measured using singlet oxygen sensor green (SOSG) and by measuring $^1O_2$ luminescence in near infrared spectral region at 1270 nm. The samples were prepared in heavy water (D_2O) and phosphate buffer solutions (PB) and irradiated using 400 nm and 460 nm wavelengths light. Exciting QD-Ce_6 complex with 460 nm light allows estimating $^1O_2$ production by QD-Ce_6 complex via energy transfer from QDs to bound Ce_6 molecules, since Ce_6 molecules do not absorb 460 nm light.
Changes in absorption and fluorescence spectra of QD, Ce_6 and QD-Ce_6 in D_2O and PB indicated that QD and Ce_6 form a complex. Irradiation of QD or Ce_6 solutions with 460 nm light did not produce any changes in SOSG fluorescence intensity, nor any $^1O_2$ luminescence signal. This indicates that no $^1O_2$ was produced. However, irradiation of QD-Ce_6 complex with 460 nm light, produced $^1O_2$ luminescence signal at 1270 nm and significantly increased intensity of SOSG fluorescence, confirming that QD-Ce_6 complex generated $^1O_2$. Our results show that QD-Ce_6 complex generates $^1O_2$. The production of $^1O_2$ occurs because of the resonant energy transfer from light absorbing QD to bound Ce_6 molecules. Therefore, QDs could be potentially used in PDT resulting in a more effective $^1O_2$ generation and cancer treatment.

P072

Biosynthesis of luminescent quantum dots in the cancer cells

Justinas Jonusas[1], Vitalijus Karabanovas[1], Ricardas Rotomskis[1,2]

[1]*Biomedical Physics Laboratory of Institute of Oncology, Vilnius University, Vilnius, Lithuania;* [2]*Biophotonics Group of Laser Research Centre, Vilnius University, Vilnius, Lithuania*

Last decade, nanometric size semiconductor particles – Quantum Dots (QD) have been heavily investigated because of their potential usage in biology and genetics as promising fluorescent probes.
Recently, organometalic synthesis techniques of QDs have been changed by the water-based synthesis. However, QDs synthesized directly in water medium does not possess high Quantum Yield (QY), have broad photoluminescence (PL) band and are highly polydisperse. Because of these reasons there is still need of biocompatible and highly controllable synthesis technique. Idea to use cells and microorganisms as biofactories for nanoparticles production has been suggested at 1989's. It was shown, that after incubation of yeast cells with cadmium salts, nanometric size particles were produced.
In this work we present possibility for biosynthesis of quantum dots in cancer cell lines. Cancer cells were incubated with cadmium chloride ($CdCl_2$) or $CdCl_2$ and selenite (Na_2SeO_3) solutions, which are used during the synthesis of glutathione capped CdSe QDs in water medium. Strong photoluminescence signal were registered from the cytoplasm of the cancer cells, after 24 hours of incubation by using spectral imaging confocal microscopy. Fluorescence lifetime imaging microscopy (FLIM) was also used to characterize optical properties of synthesized nanoparticles inside the cells. Congruence between PL spectra of

QDs synthesized in water medium and luminescence signals from cytoplasm of cancer cells have been observed after comparing the PL spectra. The obtained results propose that cancer cells can be used as biofactories for intracellular synthesis of QDs, which quality did not descend to the nanoparticles synthesized during the traditional water-based synthesis routes.

P073

Synthesis and optical characterization of rare-earth ions doped NaYF4 upconversion nanoparticles

Vitalijus Karabanovas[1], Ieva Mikalauskaite[2], Aldona Beganskiene[2], Aivaras Kareiva[2], Ricardas Rotomskis[1,3]

[1]*Biomedical Physics Laboratory, Institute of Oncology, Vilnius University, Vilnius, Lithuania, Vilnius, Lithuania;* [2]*Department of General and Inorganic Chemistry, Faculty of Chemistry, Vilnius University, Vilnius, Lithuania;* [3]*Biophotonics Group of Laser Research Centre, Vilnius University, Vilnius, Lithuania*

Recently most commonly used fluorescent probes for biological detections (organic dyes, quantum dots, fluorescent proteins) have increased concerns about their short detection times (organic dyes), cytotoxicity (quantum dots), low penetration depth and possible damage of biomolecules. Therefore, lanthanide-doped upconversion nanoparticles (UCNP) exhibiting unique fluorescent property have great potential in biomedical applications due to their immense advantages over other fluorescent probes.

Our latest investigations have focused on synthesis and characterization of the novel lanthanide-doped upconversion nanoparticles. The thermal decomposition process was chosen for the synthesis of $NaYF_4$:20% Yb, 2% Er nanoparticles. The upconversion nanoparticles were characterized using scanning electron microscopy (SEM), X-ray difraction (XRD), Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS) and luminescence spectroscopy (LS). The results of XRD indicated that cubic or hexagonal phase with high cristallinity can be obtained. The average size of nanoparticles estimated by SEM and DLS techniques depend on synthesis conditions (reaction temperature) and was found to be from ~ 30 nm to ~ 140 nm.

The shape and intensity of luminescence spectra of upconversion nanoparticles (1 wt% in toluene) under the 980 nm excitation were also investigated. Differences in upconversion luminescence spectra of different sized nanoparticles in red (650-670 nm) and green (550 nm) spectral regions were obtained. It is assumed that these differences occurs due to the change in crystalographic phase or the appearance of both (cubic and hexagonal) phases in the same sample. The possible upconversion mechanisms for Yb^{3+} and Er^{3+} doped $NaYF_4$ nanoparticles were also discussed.

P074

Synthesis and study of polyazaaromatic ruthenium (II) complexes containing 1,4,5,8-tetraazaphenanthrene and 2,2'-bipyrazine ligands for biomedical applications

Sofia Kajouj, Lionel Marcélis, Cécile Moucheron

Université Libre de Bruxelles, Bruxelles, Belgium

Platinum (II) derivatives are used for many years as anti-tumor agents [1]. However, because of their high toxicity research also focused on other transition metals including ruthenium (II). The Organic Chemistry & Photochemistry department of the ULB focused its interest on the study of polyazaaromatic ruthenium (II) complexes able to photoreact with the genetic material. It has been proved that, under illumination, an electron transfer takes place between sufficiently photo-oxidizing polyazaaromatic ruthetnium (II) complexes and one of the DNA bases, namely the guanine base [2]. The aim of this work is to prepare and characterize two novel heteroleptic complexes, namely $[Ru(TAP)_2bpz]^{2+}$ and $[Ru(bpz)_2TAP]^{2+}$, and compare their behaviour to that of the homoleptic parent compounds $[Ru(TAP)_3]^{2+}$ and $[Ru(bpz)_3]^{2+}$ [3, 4].

The bipyrazine ligand, like the 1,4,5,8-tetraazaphenanthrene, is π-deficient, but its structure makes it more flexible than its tetraazaphenanthrene analogue. The successive replacement of tetraazaphenanthrene ligands by bipyrazine ones could affect the synthesis, the stability, the photophysical and photochemical properties of the resulting complexes. Thus, the aim of this work is to determine and compare the photophysical and photochemical properties of these complexes. We show that the four complexes are able to form a photoadduct, resulting from a photo-induced electron transfer process with the guanine base. This reaction is made possible due to the exergonicity of the photo-induced electron transfer between the guanine and the photo-oxidizing complex. Regarding the photophysical properties, significant differences in the ^{3}MLCT excited states lifetimes of the four complexes are observed. It is shown that the lifetime of the complexes increases with the replacement of 1,4,5,8-tetraazaphenanthrene ligands by 2,2 '-bipyrazine.

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P075

Tropolone Derivatives as Useful Biomarkers: Large Fluorescence Enhancement Upon Binding to Biological Targets

Francisco Bosca[1], Rosa Tormos[2]

[1]*Consejo Superior de Investigaciones Cientificas, Valencia, Spain;* [2]*Universidad Politécnica de Valencia, Valencia, Spain*

Natural and synthetic tropolone derivatives have attracted considerable interest due to their multiple biological activities: antiviral, antimicrobial, and cytotoxic effects on different human tumor cell lines.[1] Colchicine (COL) and analogues such as colcemid (COD), deacetylcolchicine (DCOL), colchiceine (CEI) and deacetylcolchiceine (DCEI) are antimitotic drugs with important anticancer effects but its clinical usefulness is limited by severe toxicities. Their structure contains a tropolone moiety (C ring), in addition to a trimethoxybenzene ring (A ring) and a seven-membered ring (B ring). The binding of these drugs to tubulin is the basis for antineoplastic behavior via interrupted mitosis, and it is accompanied by a marked enhancement of the fluorescence.[2] This unusual property of the tropolone derivatives has been attributed to the immobilization and/or to the adoption of a more planar bound conformation into the cavity of this biomolecule. By contrast, when this type of study was performed with COL, DCOL and COD in the presence of other proteins such as serum albumin, trypsyn, human globin and ribonuclease, insignificant fluorescence signals were detected.[2,3] The present study explains the lack fluorescence observed in aqueous solutions of COL, DCOL and COD with proteins such as albumin as well as the emission enhancements by up to two orders of magnitude produced in some tropolone derivatives upon binding to human serum albumin (HSA). The association constants of these drugs with HSA are the basis for the results understanding. In this context, the literature contradictions about the interactions between colchicine and HSA have been unequivocally resolved. Evidences of the immobilization of these drugs into this protein have been found by UV-Vis spectrometry. Moreover, by using warfarin and ibuprofen as specific binding proves for site I and II of albumin, it has been observed that COL analogues are only bound to site II. It is also important to notice the capability of these drugs to modulate their binding properties to proteins by small changes in their structure. Hence, this study has shown tropolone derivatives fluorescence enhancements which can be used for analyze this type of drugs, as well as other

molecules that compete with them for the active sites of biomolecules.

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P076

Peroxidation of lipids photoinduced by chemically modified nanoparticles in cells and liposomes

Anna Magdalena Kozińska[1,2], A. Żądło[3], P. Łabuz[4], W. Gawlik[2], T. Sarna[1]

[1]*Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland;* [2]*Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Krakow, Poland;* [3]*Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland;* [4]*Faculty of Chemistry, Jagiellonian University, Krakow, Poland*

In this study, we analyzed the photo-peroxidation of lipids induced by two groups of nanoparticles: functionalized fullerenes and surface modified titanium dioxide. Fullerenes are a unique class of carbon molecules with interesting photophysical and photochemical properties. We analyzed a cationic C_{60} fullerene substituted with three quaternary pyrrolidinium groups (BB6). Photosensitizing properties of titanium dioxide nanoparticles can be enhanced by chemical modification of their surface using appropriate compounds. We tested the ability of two nanocrystalline materials: Hmx@qTiO_2, in which TiO_2 was modified with hematoxylin, and Brp@qTiO_2, which was treated with bromopyrogallol red, to photogenerate singlet oxygen and free radicals in selected model systems. These nanomaterials mediated photoperoxidation of lipids in POPC-cholesterol liposomes and in mouse melanoma (B16) cell culture was monitored by iodometric assay and by HPLC-Ec(Hg), which enables sensitive detection singlet oxygen specific (5α-ChOOH) and free radical dependent (7α-ChOOH, 7β-ChOOH) cholesterol hydroperoxides. Photoinduced peroxidation of lipids was also tested in the present of biological relevant such as NADH and iron ions. Moreover we analyzed the modulatory effect of endo– and exogenous melanin on the photoinduced peroxidation of lipids. We used synthetic DOPA-melanin in liposomal systems, and we used two mouse melanoma cells (B16) lines – more pigmented (F10) and less-pigmented (F0). Melanin content of the cell was obtained using EPR spectroscopy.

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P077

Hybrid systems based on zinc phthalocyanines and quantum dots

E. G. Maksimov[1], F-J. Schmitt[2], M. G. Strakhovskaya[1], D. A. Gvozdev[1], T. Friedrich[2], V. Z. Paschenko[1], A. B. Rubin[1]

[1]*M.V.Lomonosov Moscow State University, Biology Faculty, Biophysics Department, Moscow, Russian Federation;* [2]*Institute of Chemistry, Biophysical Chemistry, Berlin Institute of Technology, Berlin, Germany*

Light absorption properties of metallophthalocyanines (MPcs) are characterized by intensive Q-bands in far red region and rather low absorption of other visible light wavelengths. Photoactivation of MPcs by wider range of wavelengths may be achieved by increasing the effective absorption cross section of MPcs through energy transfer from additional light-absorbing structures. Semiconductor nanocrystals, or so-called quantum dots (QDs), absorb light in a broad optical range from ultraviolet to near infrared. The fluorescence spectrum of QDs is rather narrow, and the position of their fluorescence emission maximum can be precisely adjusted by the diameter of the nanocrystal particles. Coating of QDs provides water solubility and electrostatic interaction with other structures due to polar groups bound to the surface. It allows creating artificial light-harvesting complexes, which can serve as highly effective energy donors. Creation of hybrid structures in solution is due to electrostatic interactions, without the use of additional reagents for the formation of covalent bonds, opens up a number of promising new areas of research and corresponding applications. We have investigated the hybrid structures based on ZnPcs, bearing 8 anionic or cationic substituents, and differently charged QDs with an emission maximum at 600 nm, one with a core of CdSeCdTe/ZnS with positively charged polymer shell "poly T-APS" (QD600p) and another with a core of CdTe with negatively charged carboxyl groups (QD600n). It was shown that in a mixtures of ZnPcs and QDs, stable hybrid complexes can be formed due to electrostatic interactions. In pair of QD600p and $ZnPc^{8-}$ the efficiency of energy migration from QD to ZnPc exceeded 0.9, corresponding to ~ 30 Å distance between the donor and the acceptor. After injection QD600p we observed fast almost 140% increase of fluorescence intensity of $ZnPc^{8-}$, compared to the initial level of fluorescence. The fluorescence spectrum of the $ZnPc^{8-}$ after addition of the QD600p shifted to the red by approximately 5 nm, the fluorescence lifetimes increased by 7%. In other pairs of QDs and ZnPcs the increase of the acceptor fluorescence was insignificant. Thus, QDs can greatly expand the action spectrum of the ZnPcs that is of great interest for biological and medical MPcs applications.

P078

Paving the way towards a new photo-induced gene silencing strategy: anti-sense Ruthenium conjugates and "Seppuku" process

Lionel Marcélis, Cécile Moucheron, Andrée Kirsch-De Mesmaeker

Université Libre de Bruxelles (U.L.B.), Bruxelles, Belgium

The interest of Ruthenium(II) complexes is relatively well-known, especially thanks to their use as DNA-photoprobe and photo-sensitizers. A wide variety of ligands can be coordinated onto the Ru(II) center, which allows a fine tuning of the photophysical properties of the resulting complex. In this framework, Ru(II) complexes containing at least two π-deficient ligands (such as TAP = 1,4,5,8-tetraazaphenanthrene) are able to photoreact with the guanine base (G) via an electron transfer (ET) process, which can be followed by a covalent bond formation between the two radical species generated by this ET [1]. In order to use this photoreactivity in a gene silencing application, Ru-ASO (ASO = anti-sense oligonucleotide) conjugates have been prepared. Thus any specific sequence of DNA can be targeted by using a photoactivable Ru(II) complex conjugated to its complementary ASO. Efficient systems have been developed, which are able to block completely the enzymatic activity of polymerase and exonuclease [2].

The presence of a G base in the Ru-ASO probe sequence was first thought to be a drawback, because of competition with the photocrosslinking formation. Nevertheless, experiments have shown that the use of ASO sequences including a G (ASO_G) leads to a unique specific behaviour. These Ru-ASO_G are able to form a cyclic intramolecular product, which is obtained in presence of a non-complementary ODN sequence; or to react with its complementary target without any intramolecular side reaction. This highly selective photoreactivity has been called "Seppuku" process, as the intramolecular adduct formation prevents any side reaction in presence of a wrong target [3]. The different parameters governing this process will be presented and discussed.

This gene silencing strategy has been successfully applied to HPV (Human Papilloma Virus) infected cells. The maligant E6 gene of the HPV was silenced upon irradiation thanks to the use of a specific Ru-ASO_G conjugate. Regeneration of the native p53 protein as well as decreasing cell proliferation were observed [4]. This demonstates that the use of Ru-ASO_G and the "Seppuku"

process are an efficient and specific light-induced gene silencing strategy.

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P079

Designing New Bipyrazine Based Ru(II) Complexes for Photo-Therapeutical Applications

Kevin Garnir, Lionel Marcélis, Andrée Kirsch-De Mesmaeker, Cécile Moucheron

Université Libre de Bruxelles, Brussels, Belgium

The development of new therapeutic agents represents one of the main research objectives in the fight against cancer. [1] In this context, the Laboratory of Organic Chemistry and Photochemistry focuses its interest in the design and study of photoreactive Ruthenium II complexes. The interesting photoreactivity of these compounds is mainly due to the photooxidizing properties of complexes bearing π-deficient ligands, such as 1,4,5,8-tetraazaphenanthrene (TAP). It has been demonstrated that a photo-induced electron transfer (PET) is indeed possible between a DNA guanine moiety and a RuII complex containing at least two π-accepting ligands. [2] This primary PET process can lead to the formation of a photoadduct, in which a covalent link is formed between the RuII complex and a DNA strand.

In order to use this new kind of approach for biomedical applications, a crucial issue is the selectivity toward tumor cells and specific genes. Therefore, an original strategy using photoreactive metal complexes tethered to oligonucleotides (ODNs) has been developed by our research group. It combines the selectivity of gene therapy (based on non-covalent association between ODN strands) and the space and time control of the activity by light triggering. [3, 4]

In this context, the well known 2,2'-bipyrazine (bpz) ligand, which contains two non-chelated nitrogens inducing properties similar to those of RuII-TAP complexes, represents an interesting candidate for the design of new potential phototherapeutic RuII complexes such as [Ru(bpz)2phen]2+ (phen = 1,10-phenanthroline).

We report here the synthesis and a photo-chemical study of the new [Ru(bpz)2phen]2+ complex in order to establish its potential use in gene silencing therapy and compare its behaviour with the TAP-equivalent complex.

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P080

Singlet oxygen-sensitized delayed fluorescence of common water-soluble photosensitizers

Marek Scholz[1], Roman Dědic[1], Thomas Breitenbach[2], Jan Hála[1]

[1]*Charles University in Prague, Faculty of Mathematics and Physics, Department of Chemical Physics and Optics, Prague, Czech Republic;* [2]*University of Aarhus, Department of Chemistry, Center for Oxygen Microscopy and Imaging, Aarhus, Denmark*

Singlet oxygen ($^1O_2$), the lowest excited state of molecular oxygen, is several orders of magnitude more reactive than the ground state oxygen. It readily oxidizes a wide range of biological molecules, such as proteins, lipids, or nucleic acids. $^1O_2$ can be generated by energy transfer from light-excited triplet states of other molecules, which are referred to as photosensitizers (PS). The photosensitizing process is employed in photodynamic therapy (PDT) of cancer and other diseases, where $^1O_2$ is selectively produced in target tissues during irradiation by visible or near-infrared light. $^1O_2$ emits a very weak phosphorescence around 1275 nm, which enables its direct detection. Triplets of PSs emit a weak phosphorescence as well, often in near-infrared region. However, the detection of the weak $^1O_2$ and triplet phosphorescence is a difficult experimental task. Apart from phosphorescence, triplets of PSs may give rise to another long-lived emission – delayed fluorescence (DF). One of the mechanisms of DF generation is singlet oxygen-sensitized delayed fluorescence (SOSDF).

Our study demonstrates that a wide range of water-soluble porphyrin- and non-porphyrin-based $^1O_2$ photosensitizers for PDT (5,10,15,20-tetrakis(1-methyl-4-pyridinio) porphine (TMPyP), meso-tetrakis(4-sulfonathophenyl)porphine ($TPPS_4$), Al(III) phthalocyanine chloride tetrasulfonic acid ($AlPcS_4$), eosin Y, rose bengal, and methylene blue) exhibits SOSDF. SOSDF occurs in air-saturated water solutions of the PSs at physiological pH as well as in living 3T3 fibroblast cells incubated with the PSs. It was shown that SOSDF is the main source of DF emission in the studied systems and that the underlying mechanism consists in an encounter of triplet state of PS with $^1O_2$ molecule, giving rise to S_1 state of the PS. To this end, several experimental techniques have been employed, namely time- and spectral-resolved luminescence and triplet-triplet transient absorption. SOSDF microsecond rise-decay kinetics and overall intensity reflects lifetimes of PS triplet state and $^1O_2$ lifetime, as well as their quantum yields. SOSDF emission up to three orders of magnitude stronger than emission of $^1O_2$ infrared phosphorescence has been observed in water. The fact that SOSDF is emitted in visible spectral region also makes its detection less experimentally demanding. These features of SOSDF pave the way to noninvasive optical on-line monitoring and dosimetry of PDT in visible spectral region. Moreover, these features offer new modalities for microscopy and imaging of living cells.

P081

Label free multiphoton imaging of human pulmonary tissues through microstructured fiber or multicore image-guide

G. Ducourthial[1], C. Lefort[1], D.A. Peyrot[2], T. Mansuryan[1], S.G. Kruglik[2], Christine Vever-Bizet[2], L. Thiberville[3], F. Lacombe[4], G. Bourg-Heckly[2], F. Louradour[1]

[1]*XLIM, UMR CNRS 7252, Limoges, France;* [2]*UPMC Univ. Paris 06 FRE 3231, Laboratoire Jean Perrin and CNRS, FRE 3231, Laboratoire Jean Perrin, Paris, France;* [3]*Clinique de pneumologie, CHU Rouen and Laboratoire LITIS-QuantIF EA 4108, Rouen University, Rouen, France;* [4]*Mauna Kea Technologies, Paris, France*

Several major lung pathologies are characterized by early modifications of the extracellular matrix (ECM) fibrillar collagen and elastin network. Early diagnosis of such diseases is related to the development of innovative tools such as single[(1)] and multiphoton[(2)] confocal fibered microendoscopy. Multiphoton microscopy is of particular value for imaging ECM architecture since the combination of second-harmonic generation (SHG) and two photon excited fluorescence (2PEF) allows the simultaneous visualization of collagen (SHG) and elastin (2PEF), the main ECM proteins[(3,4)]. For this reason, multiphoton endomicroscopy is the subject of intense research developments. We report here the development of two devices, the first one using distal scanning associated to a double clad large mode area (LMA) air-silica microstructured fiber, the second one using proximal scanning of a miniature multicore image guide (30000 cores inside a 0.8 mm diameter). In both cases, the main issue has been

efficient linear and nonlinear distortion pre-compensation of excitation pulses. By inserting before the delivery fiber a compact (10 cm x 10 cm footprint) grisms-based stretcher (a grating in close contact with a prism) made of readily available commercial components, we achieved as short as 35-femtosecond-duration pulses that were temporally compressed at the direct exit of a 2-meter-long fiber[5]. Interestingly, this femtosecond pulse fiber delivery device is also wavelength tunable over more than 100 nm inside the Ti: Sapphire emission band. With the help of distal scan system, those unique features allowed us to record SHG signal of collagen and two photon excited 2PEF of both collagen and elastin. 3D imaging with 400-m-penetration depth inside the tissue was possible working with a 2-meter-long LMA fiber. With the help of proximal scanning, the miniature image guide allowed us to perform endoscopic real time microimaging of the ECM ex vivo.

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P082

Demetalation properties of chlorophyll c isolated from the diatom *Chaetoceros gracilis*

Kana Sadaoka[1], Sunao Shoji[2], Yusuke Tsukatani[2], Taichi Yoshitomi[2], Hitoshi Tamiaki[2], Yoshitaka Saga[1]

[1]Kinki University, Higashi-Osaka, Osaka, Japan; [2]Ritsumeikan University, Kusatsu, Shiga, Japan

Chlorophyll (Chl) *c*, which possesses a 17-acrylic acid residue and a porphyrin π-system, functions as light-harvesting pigments in chromophyte algae and some prokaryotes. Chl *c* is included in peripheral antenna complexes of these organisms together with Chl *a*. Metabolisms of such Chl *a*/*c*-type light-harvesting complexes are important phenomena in marine photosynthesis. However, few reports are available on metabolisms of Chl *a*/*c*-type complexes, whereas Chl *a*/*b*-type antenna complexes such as LHC II in higher plants have been extensively studied. Chemical reactivity of Chl *c* will be helpful for understanding metabolisms of Chl *a*/*c*-type complexes. From these viewpoints, we focused on demetalation of Chl *c*. Kinetic stabilities of demetalation of Chl *c* under acidic conditions were compared with protochlorophyllide (PChlide) *a* possessing the porphyrin ring and the 17-propionic acid residue, as well as chlorophyllide (Chlide) *a* possessing the chlorin ring.

Chl *c* was extracted from the diatom *Chaetoseros gracilis*. PChlide *a* and Chlide *a* were prepared from naturally occuring Chl *a*. These pigments were purified by reverse-phase high-performance liquid chromatography, and were demetalated in a mixture of acetone and water (3/1, vol/vol) containing hydrochloric acid under the control of a reaction temperature at 25 °C. Kinetic analysis was performed by monitoring Soret absorbances of the three chlorophyllous pigments.

The 445-nm Soret absorption band of Chl *c* gradually decreased and a new absorption band appeared at 428 nm. From the temporal absorbance changes at the Soret peak positions, pseudo-first-order demetalation rate constants were estimated. The demetalation rate constant of Chl *c* was smaller than that of PChlide *a* under the same conditions. The difference was ascribable to the substitution at the 17-position: the unsaturated 17^1-17^2 bond was responsible for the slow demetalation kinetics of Chl *c*. This is consistent with the relationships between 3-vinyl- and 3-ethyl-Chls in previous reports,[1,2] in which the unsaturated 3-vinyl group slowed down the demetalation kinetics.

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P083

Aluminium phthalocyanine nanoparticles for fluorescence diagnostics

Jasmin Breymayer[1], Angelika Rück[1], Victor B. Loschenov[2], Rudolf Steiner[1]

[1]Institute for Lasertechnologies Ulm, Ulm, Germany; [2]A.M. Prokhorov General Physics Institute RAS, Moscow, Russian Federation

Aluminium phthalocyanine (AlPc) does not fluoresce in the form of nanoparticles (nAlPc) but in its molecular form. Previous investigations (Vasilchenko et al. 2010) indicated an interaction between inflammatory processes and separation of AlPc monomers from the nanoparticles. We analyzed the influence of monocytes/macrophages and skin cells and their cocultivation on these nanoparticles by fluorescence microscopy and flow cytometry.

P084

FLIM for the characterization of sensitized tissues in rheumatoid arthritis model

Romualdas Rudys[1], Jurga Papečkienė[2], Saulius Bagdonas[1], Gailutė Kirdaitė[2], Ričardas Rotomskis[1]

[1]Biophotonics Laboratory, Laser Research Center, Vilnius University, Vilnius, Lithuania; [2]State Research Institute Center of Innovative Medicine, Vilnius, Lithuania

Medical applications inspire an increasing interest in exploiting tissue autofluorescence and sensitized fluorescence for non-invasive clinical diagnosis and research. Numerous fluorophores found in tissues mostly possess fluorescence lifetimes in the range of 100 ps to 10 ns. Fluorescence lifetime imaging (FLIM) technique relies on the fluorescence lifetime of a fluorophore, which depends on the micro-environmental characteristics, such as viscosity, pH and oxygen concentration. Compared to conventional fluorescence microscopy, which is based on the spectral features and intensity, fluorescence lifetime is independent of the excitation power and fluorophore concentration providing additional possibilities for visualization. As a result, fluorescence microscopy combined with fluorescence lifetime measurements can serve to distinguish fluorophores in specific tissues, organs, as well as to detect their changes in pathological situations. Dominant tissue autofluorescing substances in the red spectral region are porphyrins, more precisely, 5-aminolevulinic acid (ALA)-induced protoporphyrin IX (PpIX), produced in cells via the heme synthesis pathway. Since the inflamed synovium in the case of rheumatoid arthritis (RA), a chronic inflammatory disease of the joints, exhibits many features typical for neoplastic tissues, there are numerous attempts to employ endogenous porphyrins for diagnostic and therapeutical purposes in rheumatology as well.

This study presents preliminary investigations on fluorescence morphology and FLIM aimed to distinguish structural features and to detect the accumulation of endogenous porphyrins in synovium and cartilage tissues using the experimental model of rheumatoid arthritis.

To measure the lifetimes a pulsed diode laser (20 MHz, 405 nm) (PDL 800-B, PicoQuant GmbH) was coupled to a laser scanning microscope Nikon “Eclipse TE2000”. FLIM was performed using a single-channel time correlated single photon counting (TCSPC) module PicoHarp 300. Fluorescence lifetime was registered with a single channel SPAD detection unit at 650±75 nm spectral range.

The analysis of FLIM imaging data of synovium and cartilage tissues allows distinguishing healthy and inflamed tissues considering their distinctive autofluorescence lifetimes. A presence of several endogenous porphyrins with different fluorescence lifetimes has been revealed in the sensitized inflamed rabbit knee synovium and cartilage specimens and their intratissual distribution was compared with morphological data.

P085

Fluorescein dodecyl ester as protonophore providing submicromolar-range uncoupling of oxidative phosphorylation in mitochondria

Elena A. Kotova, Maria M. Shchepinova, Stepan S. Denisov, Lyudmila S. Khailova, Dmitry A. Knorre, Galina A. Korshunova, Fedor F. Severin, Yuri N. Antonenko

A.N.Belozersky Institute of Phys.-Chem. Biology, M.V. Lomonosov Moscow State University, Moscow, Russian Federation

Uncouplers are low molecular weight compounds which are capable of carrying protons across inner mitochondrial membrane thereby uncoupling oxidation and phosphorylation. Current interest in this class of compounds is associated with their ability to protect cells from damage in a number of physiological models leading to the idea of their use in therapy. To facilitate the study of the distribution of uncouplers in tissues, it is helpful to work with agents having bright fluorescence. In search of fluorescent uncouplers, two esters of fluorescein, n-butyloxycarbonyl-fluorescein (C_4-FL) and n-dodecyloxycarbonyl-fluorescein (C_{12}-FL), were synthesized and characterized. The growth of polarization with increasing lipid concentration, which apparently reflected the dye binding to liposomes, was observed only with C_{12}-FL, but not with C_4-FL and unaltered fluorescein. Importantly, C_{12}-FL was shown to induce proton permeability in lipid membranes, while C_4-FL was inactive. The pK_a of C_{12}-FL was estimated to be 7.5. C_{12}-FL, but not C_4-FL, increased the respiration rate and decreased membrane potential of isolated rat liver mitochondria with a half-maximal effective concentration of 500 nM. In wild-type yeast cells, C_{12}-FL localized predominantly in plasma membranes, while in AD-8 mutant cells, lacking MDR pumps, C_{12}-FL stained cytoplasmic organelles with some preference for mitochondria.

P086

Time-resolved fluorescence spectroscopy of protoporphyrin IX and water soluble porphyrins in model biological systems and in sensitized tissues

Saulius Bagdonas, Romualdas Rudys, Ricardas Rotomskis

Vilnius University Laser Research Center, Vilnius, Lithuania

The measurements of the fluorescence intensity of endogenous porphyrins produced in biological tissues after application of 5-aminolaevulinic acid (ALA) or its derivatives are usually performed for diagnostic and therapeutic purposes during routine procedures of the photodynamic therapy (PDT). Sometimes the dominant fluorescence of protoporphyrin IX is accompanied by the additional red fluorescence originated from other porphyrins formed during the haem synthesis, discrimination of which requires application of additional spectroscopic techniques.

The study is focused on the steady state spectroscopy and fluorescence lifetime measurements of protoporphyin IX and less hydrophobic uro- and copro- porphyrins. The analysis of the spectral data and the fluorescence decay kinetics obtained for exogenous porphyrins in model biological media will be performed in comparison with the corresponding data being measured in the suspensions of sensitized cells and tissues with an aim to estimate the impact of the microenvironment on the spectral properties characterizing different porphyrin species. The determined fluorescence lifetime values will be used for the interpretation of the images taken during fluorescence lifetime imaging microscopy (FLIM) measurements. The possibilities to discriminate between different species of endogenous porphyrins present in the specimens of sensitized knee synovium and cartilage tissues after an intraarticular injection of ALA and ALA-Me will be demonstrated in the case of the experimental model of rheumatoid arthritis.

P087

Synthesis, spectroscopic and photophysical characterization of a novel CF_3 and I groups bearing phthalocyanine photosensitizers

Łukasz Łapok[1], Arkadiusz Gut[1], Mariusz Kępczyński[1], Grzegorz Szewczyk[2], Tadeusz Sarna[2], Maria Nowakowska[1]

[1]Faculty of Chemistry, Jagiellonian University, Kraków, Poland; [2]Department of Biophysics, Jagiellonian University, Kraków, Poland.

There is a need for a new cancer treatment technologies that would be less invasive and would possess reduced side effects. One of such promising therapeutic procedures for the management of a variety of tumors is photodynamic therapy (PDT). Photodynamic therapy (PDT) is a clinically approved method for treatment of cancer, microbial infections, and some other diseases. It involves formation of cytotoxic singlet oxygen, produced upon light induced activation of photosensitizer molecule. A great deal of attention has been paid by researchers to develop next generation photosensitizers that would possess ability to absorb light from the visible and near-IR region of the spectrum while maintaining other parameters important for PDT, such as photostability, lack of dark cytotoxicity or high singlet oxygen quantum yield. In one of our current projects, we developed an interest in phthalocyanines as PDT active agents. Phthalocyanines seem to be better suited, more than any other group of organic dyes, as perfect candidates as photosensitizers in PDT. This is mainly due to their intrinsic properties, viz. efficient absorption of light in the visible and IR region, high molar extinction coefficients, good photochemical stability, and above all, an ability to produce efficiently reactive oxygen species (ROS). We report here the synthesis, spectroscopic characterization and photophysical properties of two novel phthalocyanines, viz. zinc 1,8(11),15(18),22(25)-tetraiodo-3,10(9),17(16),24(23)-tetra-*tert*-butylphthalocyanine ($ZnPcI_4$(t-Bu)$_4$) and zinc 1,8(11),15(18),22(25)-tetra-(trifluoromethyl)-3,10(9),17(16),24(23)-tetra-*tert*-butylphthalocyanine ($ZnPc(CF_3)_4$(t-Bu)$_4$). Both compounds were obtained via reaction of a corresponding phthalonitriles, that is 5-tert-butyl-3-iodophthalonitrile and 5-tert-butyl-3-(trifluoromethyl) phthalonitrile, with a zinc acetate at elevated temperature in 2-dimethylaminoethanol. Both phthalocyanines are characterized by a very low fluorescence quantum yield (Φ_f): 0.03 for $ZnPcI_4$(t-Bu)$_4$ and 0.02 for $ZnPc(CF_3)_4$(t-Bu)$_4$. High singlet oxygen quantum yields were measured for obtained photosensitizers: 76% for $ZnPcI_4$(t-Bu)$_4$ and 98% for $ZnPc(CF_3)_4$(t-Bu)$_4$. Moreover, they exhibit strong absorption of light in the visible region of the spectrum (640-700 nm) with very high molar extinction coefficients. Thanks to the presence of bulky tert-butyl groups attached to the peripheral positions these phthalocyanines show lack of aggregation.

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Photogenotoxicity induced by reactive chlorpromazine metabolites

Inmaculada Andreu[1], Fabrizio Palumbo[2], Daniel Hernández[1], María Dolores Coloma[1], José Vicente Castell[1], Miguel Angel Miranda[2]

[1]Unidad Mixta de Investigación IISLaFe-UPV, Hospital La Fe, Valencia, Spain; [2]Instituto Universitario Mixto de Tecnología Química UPV-CSIC, Valencia, Spain

Metabolites may be more toxic and reactive than the parent drug. Upon irradiation, they can generate reactive intermediates

capable of binding to key macromolecules such as DNA. Therefore, identification of metabolites with phototoxic or adduct forming capability is a major challenge. Chlorpromazine (CPZ) and related phenothiazine drugs, used as psychotropic agents in humans, mediates photosensitivity side effects. Hence, the aim of the present work is to assess the photogenotoxic potential of chlorpromazine metabolites. Here, the photophysical properties of CPZ metabolites have been studied in organic and aqueous medium. As in the case of parent CPZ, laser flash photolysis experiments show the presence of two transient species, triplet and radical cation, with maxima centered at 480 and 520 nm, respectively.

To investigate the molecular mechanism involved in UVA-induced DNA damage, experiments with CPZ and its metabolites, in the presence of supercoiled DNA, were carried out. Thus, conversion of form I into form II was observed, indicating formation of single-strand breaks. In order to reveal the nature of damages induced on the DNA bases, repair enzymes (formamidopyrimidine glycosylase, E.coli endonuclease III and T4 endonuclease V) have been used. Interestingly, chlorpromazine metabolites showed higher photogenotoxicity than CPZ itself in all cases.

P089

Fluorescent Gold Nanoclusters for dual X-Ray-Optical imaging

Akvile Slektaite[1], Reda Kubiliute[2], Ricardas Rotomskis[2]

[1]*Faculty of Natural Sciences, Vilnius University, Vilnius, Lithuania;* [2]*Biomedical Physics Laboratory of Institute of Oncology, Vilnius University, Vilnius, Lithuania*

Fluorescent imaging is one of the main imaging methods in biological studies, however it is still not fully adapted in medical practice. Main drawbacks of this method are limited examination depth in living tissue and imperfect characteristics of exogenous fluorescent agents applied. In biological studies most commonly used fluorophores are organic dyes and semiconductor quantum dots. Unfortunately organic dyes provide poor photostability, small Stoke's shifts, while quantum dots are proven to be toxic, and have to be coated with biocompatible materials before using in biological environments.

Nowadays the key method in medical diagnostics is X-ray imaging. This method is based on biological tissue natural X-ray contrast, but often needs an additional contrast enhancement by exogenous contrast media. Currently used iodine-based contrast agents sometimes lead to toxic effects in kidneys, also have short blood circulation half-life and cannot be functionalized for selective targeting.

Here we propose dual-modality probes – fluorescent gold nanoclusters (AuNCs), which could be suitable for both fluorescent and X-Ray imaging. These imaging agents could overcome discussed drawbacks and bring optical methods closer to everyday medicine. Fluorescent AuNCs show great promise in fluorescence imaging due to their small size (less than 2 nm), good quantum yield, and stability. Also due to the high atomic number of gold, these probes provide much higher X-ray contrast intensity than iodine based agents. Generally AuNCs are non-toxic and offer a wide range of sizes, shapes and surface coating choices. In addition, particles can be functionalised with biologically active ligands, which could allow selective accumulation in tumour tissue and lead to specific diagnostics.

In our study, we present synthesis method of AuNCs using morpholine ligands as stabilization agents. These nanoclusters yield stable fluorescence emission in visible spectrum range, though its intensity strongly depends on pH value of the reaction mixture. Also AuNCs possess X-ray absorption properties comparable to standard contrast media. Sufficiently concentrated these particles were injected into a live mouse and tracked in animal body with standard X-Ray system.

Our results suggest that fluorescent gold nanoclusters could be suitable for dual X-ray-Optical imaging and therefore could improve medical diagnostics.

P090

An *in vivo* fluorescence study of the photomotile ciliate *Blepharisma japonicum* albino strain

Giovanni Checcucci, Francesco Ghetti, Ranieri Bizzarri

CNR Istituto di Biofisica, Pisa, Italy

The heterotrichous ciliate *Blepharisma japonicum* shows a photomotile reaction (step-up photophobic response) enabling it to avoid lighted areas [1]. This behaviour is elicited by the absorption of light by blepharismin [2], a benzodianthronic molecule mainly confined in numerous submembranal pigment granules, but also largely present in the cilia and other parts of the cell body in such a great amount that the microorganism is red coloured. Blepharismin has also a defensive function against predators [3] because of its photosensitizing properties, which are the cause of the photoavoiding reaction too.

We are running a series of *in vivo* fluorescence studies by means of confocal microscopy with the aim of analyse blepharismin photophysical determinants (e.g., emission spectra profile and fluorescence lifetime emission) and correlate them with its different locations in the cell body and possibly with its functional properties.

However, since the sheer amount of blepharismin in wild type cells may hinder this analysis, we have started a parallel study in an albino strain of *B. japonicum*, characterized by a very low content of blepharismin, but still capable to react to light.

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