Società Italiana
di Fotobiologia

Symposium Augustanum
Società Italo-Tedesca
di Dermatologia

Gruppo Italiano di
Fotodermatologia
SIDeMaST

Joint Meeting

Photobiology and Phototherapeutic Techniques Oxidative Reactions, Damages and Therapeutical Effects

Grand Hotel & La Pace
Montecatini Terme (Italy), May 11th – 13th 2006

President
Prof. Torello Lotti, MD

Co-Presidents
Pier Giacomo Calzavara-Pinton, MD
Francesco Ghetti
Prof. Michael Meurer, MD

Programme and Abstracts

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GENERAL INFORMATION

Meeting Venue
Grand Hotel & La Pace
Via della Torretta, 1
51016 Montecatini Terme (PT)
Phone +39 0572 9240
Fax +39 0572 78451

Official language
The official Conference language will be English

ECM credits
Application has been forwarded for both National and European credits.
This event has been accredited by the Italian Ministry of Health with 14 credits E.C.M.

Registration fees

Participant	Before April 1st, 2006	After April 1st , 2006	On-site
Participant > 30 y.o	Euro 300	Euro 350	Euro 400
Participant < 30 y.o	Euro 200	Euro 250	Euro 300

Registration fee for participants includes
Congressual kit • Abstracts Book • Admission to Plenary Sessions • Coffee breaks (Friday morning, 12th and Saturday morning, 13th, May 2006)

Registration fee for participants does not include
Technical Practical Course • Lunches.

TECHNICAL PRACTICAL COURSES

• May 12th, 2006 > h 12.30 - 14.30 Euro 200
"Laser" Practical Course

• May 12th, 2006 > h 17.40 - 19.00 Euro 200
Practical Course "The Treatment of Vitiligo"

• May 13th, 2006 > h 12.30 - 14.00 Euro 200
Technical Practical Course "PDT"

PROGRAMMA

Thursday, May 11th, 2006

14.45 - 15.00 **Opening** T. Lotti (Italy)

Chairs: J. Hawk (United Kingdom), J. Hercogova (Czech Republic)

15.00 - 15.30 Opening Lecture "Sun, Genes and Melanoma" *K. Wolff (Austria)*

15.30 - 16.00 Reactive oxygen species: a tale of life and death *G. Ambrosio (Italy)*

16.00 - 16.30 Oxidative stress in human epidermis *K. Schallreuter (United Kingdom)*

16.30 - 17.00 Ultraviolet therapy: when and how *G. Leone (Italy)*

17.00 - 17.30 Photoprotection: methods and tools *J. Hawk (United Kingdom)*

17.30 - 18.00 Campaigns for photoprotection: did we win the battle? *J. Hercogova (Czech Republic)*

18.00 - 19.30 ROUND TABLE:
Psoriasis and biological therapies: state of the art and new proposals in Tuscany
Scientific Coordinator: T. Lotti (Italy)
Chairs: B. Giannotti (Italy), P. Fabbri (Italy), P. Barachini (Italy)
Topics:
Differentiation of the biological therapies in the treatment of psoriasis *M. Ceccarini (Italy)*
Clinical experience in the treatment of psoriasis *P. Martini (Italy)*
Clinical experience in the treatment of the psoriasic arthritis *M. Matucci Cerinic (Italy)*
National and International guidelines in the treatment of psoriasis *M. Fimiani (Italy*)

Friday, May 12th, 2006

08.30 - 09.00 **Plenary Session: Podium discussion of Posters**

Plenary Session: Symposium Augustanum
Chairs: B.R. Balda (Germany), L. Andreassi (Italy)

09.00 - 09.20 Chemical markers of pheomelanin: evaluation of their potential of predicting individual UV susceptibility *A. Napolitano (Italy)*

09.20 - 09.40 The role of oxidative stress in skin photodamage *M. Wlashek (Germany)*

09.40 - 10.00 Epidermal pathophysiology of antioxidants *M. Podda (Germany)*

10.00 - 10.20 Cell cycle checkpoints: guarding the genome against photodamage *T. Herzinger (Germany)*

10.20 - 10.40 Therapeutic mechanism of ECP: possible relation with oxidative stress *M. Fimiani (Italy)*

10.40 - 11.00 Extracorporeal photophoresis in the treatment of oral lichen planus and chronic graft vs host disease *C. Kunte (Germany)*

11.00 - 11.20 Advances and new technologies in the preparation of sunscreens *C. Anselmi (Italy)*

11.20 - 11.40 Clothing photoprotection *K. Hoffmann (Germany)*

11.40 - 12.00 Systemic vs microphocused UV therapy *T. Lotti (Italy)*

12.00 - 12.30 **General Assembly Meeting of the German-Italian Dermatological Society**

12.30 - 14.30 **"Laser" Practical Course** *I. Ghersetich (Italy), N. Zerbinati (Italy), L. Marini (Italy)*
Plenary Session

Chair: G. Canti (Italy)

14.30 - 15.00 Potential protection of skin by acute UVA irradiation – from cellular to animal models *R. Tyrrell (United Kingdom)*

15.00 - 15.20 Autofluorescence spectroscopy for characterization of histological and biochemical tissue properties *G. Bottiroli (Italy)*

Plenary Session: Symposium ESPD
Chairs: G. Leone (Italy), G. Murphy (Ireland)

15.20 - 15.40 Cellular and molecular events leading to development of skin cancer *H.N. Ananthaswamy (USA)*

15.40 - 16.00 Molecular approaches to photoprotection *P. Schröder (Germany)*

16.00 - 16.20 Systemic photoprotection with antioxidants *M. Picardo (Italy)*

16.20 - 16.40 New strategies in topical sunscreen use *G. Murphy (Ireland)*

16.40 - 17.00 Molecular aspects of oxidative stress *G. Palumbo (Italy)*

17.00 - 17.20 Prevention of photodermatoses: the role of oxidative stress *A. Pacifico (Italy)*

17.20 - 17.40 Photorejuvenation: what's new? *I. Ghersetich (Italy)*

17.40 - 19.00 **Practical Course "The Treatment of Vitiligo"** *T. Lotti (Italy), L. Andreassi (Italy)*

Saturday, May 13th, 2006

08.30 - 09.00 **Plenary Session: Podium discussion of Posters**

Plenary Session: Workshop GIFDE

09.00 - 09.20 Modern treatment of Solar Urticaria *S. Beissert (Germany)*

09.20 - 09.40 Photosensitivity and photoprovocation in cutaneous LE *A. Kuhn (Germany)*

09.40 - 10.00 PDT update 2006 *P.G. Calzavara-Pinton (Italy)*

10.00 - 10.20 PDT with systemic sensitizers *G. Roncucci (Italy)*

10.20 - 10.40 Oxidative damage in Vitiligo *B. Ortel (Austria)*

10.40 - 11.00 Applications of photodynamic therapy in the treatment of microbial infections *G. Jori (Italy)*

11.00 - 12.00 GIFDE: free communications *P.G. Calzavara-Pinton (Italy), T. Lotti (Italy), K. Janowski (Poland)*

12.00 - 12.30 Metalloproteinases and their inhibitors: a lesson for the Photobiologist *I. Bertini (Italy)*

12.30 - 14.00 **Technical Practical Course "PDT"** ***R. Rossi (Italy), P. G. Calzavara-Pinton (Italy)***

Plenary Session: SIF Congress
Chair: F. Ghetti (Italy)

14.00 - 17.30 Free communications

17.30 - 17.45 Closing Remarks T. Lotti (Italy)

Abstracts

SUN, GENES AND MELANOMA

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

UV radiation represents only 4 % of the solar spectrum but this small portion is responsible for skin damage and skin cancer. Accumulation of UV effects starts in childhood and the effect of a UV dose that has once hit the skin can never be reversed. UVA and UVB induce molecular reactions even without erythema and DNA damage occurs also below erythema threshold doses. The only known etiopathogenic agent in melanoma oncogenesis is ultraviolet radiation (UVR) but how UVR induces melanoma is not known. The incidence of melanoma worldwide is increasing and at current rates one in 62 white Americans has a life time risk of developing invasive melanoma. One person dies of melanoma every 68 minutes and in 2005 close to 8,000 deaths have been attributed to melanoma in the USA. Melanoma risk is strongly related to the number of nevi and the number of nevi increases in people with high levels of sun exposure and episodes of sun burn. Pathways of UV carcinogenesis include DNA damage, gene mutation leading to abnormal cell proliferation and immunosuppression permitting uninhibited proliferation of abnormal cells.

UV-induced mutations disable genes of cell cycle regulation and these pertain both to proto-oncogenes (e.g. Ras) and tumor suppressor genes (e.g. p53). The dysfunction of these genes can cause malignant transformation. Genes involved in melanoma oncogenesis are, amongst others, INK 4a/p16, INK 4a/pARF, CDK4/p16, p53, PTEN/MMACI, Ras, c-myc, β-catenin and MRC-I. There is no single "melanoma gene" and the most important single risk rendering an individual susceptible to melanoma oncogenesis is the skin photophenotype I. Nonetheless, genes involved in melanoma oncogenesis have been targeted in corrective gene therapy, for instance, employing a BCL-2 antisense strategy or novel Ras antagonists.

A host of surface molecules, their ligands and cytokines have been shown to play role in the progression of a normal melanocyte to a benign proliferative melanocytic process and from here to an in situ primary melanoma, tomorigenic primary melanoma and metastases. Based on the observation that reverse transcriptase activity can be induced in melanoma cells by UV radiation a novel human endogenous retrovirus, named MERV-1, has been found in human melanomas. It has a high homology to the only other known human endogenous retrovirus HERV-K. MERV-1 possesses all the complements of a mature retrovirus including gag, corf and env. MERV-1 is present in primary and metastatic melanomas but not in normal melanocytes and benign nevi. How MERV-1 is involved in melanoma oncogenesis is not yet known but a serologic response to MERV-1 antigens does occur in melanoma patients.

REACTIVE OXYGEN SPECIES. A TALE OF LIFE AND DEATH

Giuseppe Ambrosio
Division of Cardiology, University of Perugia School of Medicine, Perugia, Italy

Pathological conditions that predispose to cardiovascular events, such as hypertension, hypercholesterolemia, and diabetes, are associated with oxidative stress. These observations and further data derived from a plethora of investigations provided accumulating evidence that oxidative stress is decisively involved in the pathogenesis of endothelial dysfunction and atherosclerosis. Several enzymes expressed in vascular tissue contribute to production and efficient degradation of reactive oxygen species, and enhanced activity of oxidant enzymes and/or reduced activity of antioxidant enzymes may cause oxidative stress. Various agonists, pathological conditions, and therapeutic interventions lead to modulated expression and function of oxidant and antioxidant enzymes, including NAD(P)H oxidase, endothelial nitric oxide synthase, xanthine oxidase, myeloperoxidase, superoxide dismutases, catalase, thioredoxin reductase, and glutathione peroxidase. Data from numerous studies underline the importance of dysregulated oxidant and antioxidant enzymes for the development and progression of atherosclerotic disease in animal models and humans. Specific pharmacological modulation of key enzymes involved in the propagation of oxidative stress rather than using direct antioxidants may be an approach to reduce oxygen radical load in the vasculature and subsequent disease progression in humans.

ULTRAVIOLET THERAPY: WHEN AND HOW

Giovanni Leone
Phototherapy Unit, S. Gallicano Institute, IRCCS, Rome, Italy

Within the last two decades, phototherapy has turned out to be a major therapeutic strategy in dermatology and thus has significantly influenced the treatment of many dermatoses. To date, the aims of phototherapy are the suppression of ongoing disease processes and the prevention or modulation of pathogenetic mechanisms causing the disease.

Increasing knowledge about the effects of UV radiation on the skin, with or without photosensitizing agents, has led to the development of new forms of photo(chemo)therapy. These allow good therapeutic results to be achieved in the treatment of not only psoriasis, but also other chronic inflammatory skin diseases. Such advances in the field of photobiology led to the development of different delivery systems for phototherapy such as narrowband UVB, targeted UVB phototherapies and UVA1 phototherapy. The effectiveness of these new sources have given us additional insight into how phototherapy works with minimal side effects in regards to treatment of several skin diseases that classically were not treated with phototherapy.

This overview aims to summarize the known biological and therapeutical effects induced by different phototherapeutic modalities that are currently used to treat numerous skin diseases.

PHOTOPROTECTION: METHODS AND TOOLS

John Hawk
Photobiology Unit , St John's Institute Of Dermatology
St Thomas' Hospital, London SE1 7EH, United Kingdom

Cutaneous photoprotection is the avoidance of unwanted ultraviolet, rarely visible, radiation-induced skin effects through the use of preventive measures. These effects may be either the normal adverse events potentially occurring in all exposed subjects, such as sunburn, photoageing or photocarcinogenesis, or the abnormal outcomes affecting up to a fifth of subjects at higher latitudes, namely the photodermatoses. These latter comprise the idiopathic, probably immunological conditions (polymorphic light eruption, actinic prurigo, hydroa vacciniforme, chronic actinic dermatitis and solar urticaria), the DNA repair-defective disorders (especially xeroderma pigmentosum), chemical and drug photosensitivity, whether from exogenous drugs or endogenous porphyrins (leading to the hepatic and erythropoietic porphyrias, particularly porphyria cutanea tarda and erythropoietic protoporphyria), and the ultraviolet radiation (UVR)-exacerbated dermatoses (such as psoriasis, eczema and lupus erythematosus). For all these, protective measures in order of reliability include UVR avoidance, exposing the skin to sunlight only at times when its disorder-inducing wavelengths are relatively weak, wearing UVR-protective clothing and using topical sunscreens, while for the photodermatoses, taking or applying medications against the unwanted disorder is a further, often effective option. Sunscreens are the most controversial photoprotective method, seeming often in epidemiological studies to be ineffective against photocarcinogenesis, but if high protection preparations are used liberally, carefully and often, the evidence is strong that they are very helpful. However, such careful use is difficult, and sunscreens should only be employed as a supplement to the other suggested measures.

CHEMICAL MARKERS OF PHEOMELANIN: EVALUATION OF THEIR POTENTIAL OF PREDICTING INDIVIDUAL UV SUSCEPTIBILITY

Alessandra Napolitano,
Department of Organic Chemistry and Biochemistry, University of Naples Federico II; Naples Italy

Red hair, fair skin and the lack of tanning ability are generally recognized as risk factors for melanoma and other skin cancers under conditions of prolonged solar exposure. These pigmentary traits are associated with some loss of function mutations at the melanocortin-1 receptor which cause the melanocyte to produce red photosensitizing pheomelanin in preference to the default pigment, the dark eumelanin. At present evaluation of UV susceptibility relies exclusively on determination of the phenotype and phototype. Yet, not all phenotypically similar red haired individuals exhibit the same erythemogenic responses and tanning capacities, suggesting that the type and levels of pheomelanin may profoundly affect the individual response to UV radiation. Identification of specific markers of pheomelanin pigments and determination of possible relationships with skin phototypes and UV susceptibility appear therefore attractive goals. Recently we developed new procedures for analysis of pheomelanin based on identification and quantitation of specific structural markers obtained by chemical degradation of hair. The levels of these structural markers , *viz.*1,3-thiazole-2,4,5-tricarboxylic acid (TTCA) and 6-(2-amino-2-carboxyethyl)-2-carboxy-4-hydroxybenzothiazole (BTCA) were determined in groups of red hair individuals. Whereas the majority of the red hair samples afforded TTCA in variable yields, only a restricted number of samples gave BTCA. Herein, we report new data from a larger group of red haired individuals (n=22). As a rule, the lowest MED and 5-days delayed pigmentation values were associated with BTCA-positive individuals while TTCA-positive subjects gave higher MED values (mean value 67.5 mJ cm^{-2}, $p< 0.001$). Overall, these results hint at pheomelanin marker quantitation, in combination with genetic analysis and photobiogical parameters, as potential means for routine prediction of high risk individuals. A possible association of oxidative stress conditions with pheomelanic pigmentation will also be discussed.

THE ROLE OF OXIDATIVE STRESS IN SKIN PHOTODAMAGE

Meinhard Wlaschek and Karin Scharffetter-Kochanek
Department of Dermatology and Allergic Diseases, University of Ulm, Germany

The skin is increasingly exposed to ambient UV-irradiation thus increasing its risk for photooxidative damage with longterm detrimental effects like photoaging, which is characterized by wrinkles, loss of skin tone and resilience. Photoaged skin displays prominent alterations in the cellular component and extracellular matrix of the connective tissue with a severe loss of interstitial collagens, the major structural proteins of the dermal connective tissue, and with an accumulation of disorganized elastin in the deep dermis. The unifying pathogenic agents for these changes are UV-generated reactive oxygen species (ROS) which deplete and damage non-enzymatic and enzymatic antioxidant defense systems of the skin. As well as causing permanent genetic changes, ROS activate cytoplasmic signal transduction pathways in resident fibroblasts that are related to growth, differentiation, senescence and connective tissue degradation. This lecture focuses on the role of UV-induced ROS in the photodamage of the skin resulting in clinical and biochemical characteristics of photoaging. In addition, the relationship of photoaging to intrinsic aging of the skin will be briefly discussed. A decrease in the overall ROS load by efficient sunscreens or other protective agents may represent promising strategies to prevent or at least minimize ROS induced photoaging.

CELL CYCLE CHECKPOINTS: GUARDING THE GENOME AGAINST PHOTODAMAGE

T. Herzinger

At key transitions during the cell division cycle, signaling pathways monitor the successful completion of upstream events prior to proceeding to the next phase. These regulatory pathways are commonly referred to as cell cycle checkpoints. Inactivation of cell cycle checkpoints is a major cause of genomic instability and aneuploidy in sporadic cancer and genetic cancer susceptibility syndromes. Cells arrest at these checkpoints temporarily to prevent replication of damaged DNA templates or segregation of broken chromosomes until the damage has been repaired. Checkpoint signaling may also result in activation of pathways leading to programmed cell death if the damage cannot be properly repaired. There are at least three checkpoints at critical cell cycle transitions that allow for the repair of DNA damaged by ultraviolet radiation or other genotoxic agents: at the G1/S transition, during S-phase and before the entry into mitosis (G2/M). Cell cycle checkpoints are also activated by UV-phototherapy and PUVA, and contribute to the anti-proliferative action of these treatments.

THERAPEUTIC MECHANISM OF ECP: POSSIBLE RELATION WITH OXIDATIVE STRESS

M. Fimiani, G.B. De Aloe and P. Rubegni
Dpt. Medicina Clinica e Scienze immunologiche – Sez. Scienze Dermatologiche

Extracorporeal photochemotherapy (ECP) is an original procedure introduced by Edelson in 1987 to treat Sèzary syndrome and consisting in an extracorporeal exposure of PBMC to photoactivated 8-MOP and subsequent return to the patient. Besides cutaneous T-cell lymphoma (CTCL), ECP is used in the treatment of several autoimmune T-cell mediated diseases such as scleroderma, pemphigus vulgaris, LES and acute and chronic GvHD. One of the most intriguing aspect of ECP is its ability to induce two apparently opposed effects: activation of the immune system against neoplastic cells (as in CTCL) and down regulation of the activity of T-cell clones in autoimmune diseases (as in systemic sclerosis, systemic lupus eritematosus and pemphigus vulgaris) and auto-allogenic immune responses (as in GVHD and allograft rejection) . Some recent clinical and experimental investigations seem to support the hypothesis that the peculiar immunomodulating effects of ECP depend on its ability to induce two main events: PUVA-related damage of T-lymphocytes and differentiation of monocytes into active dendritic antigen presenting cells. Moreover, there is growing evidence to suggest that material derived from apoptotic cells, once phagocyted, does not just quietly disappear but actively modulates the immune system in a way which is not yet completely elucidated. Summing up ECP therefore seems to have at least six main immunological activities: massive induction of lymphocyte apoptosis (about 80% of phototreated cells); fast promotion of monocyte differentiation up to the stage of immature actively phagocyting DCs; possibly slower progressive maturation of immature DCs into active APCs; strong stimulation of monocytes to produce increasing amounts of cytokines (IL-10, IL-6, TNFα); induction of regulatory T-cell subsets synthesizing IL-10; and in the long period restoration of altered DC1/DC2 and Th-1/Th-2 balance. However it is puzzling how ECP can have apparently opposite effects in different clinical conditions such as CTCL and cGVHD. Since it is well known that interactions between apoptotic lymphcytes and DCs can have both tolerogenic and stimulatory effect, it is likely that the modalities of the apoptotic pathway, the type and functional status of DCs and their precursors, as well as the amount and activities of stimuli (Th1/Th2 environment, interleukins, size and number of treated clones, therapy) affecting function are the majour elements conditionating the type of immune response.

EXTRACORPOREAL PHOTOPHORESIS IN THE TREATMENT OF ORAL LICHEN PLANUS AND CHRONIC GRAFT VS HOST DISEASE

Kunte C, Michelsen S, Plewig G
Department of Dermatology, Ludwig-Maximilians-University of Munich

Erosive oral lichen planus is typically therapy-resistant. Histologically and immunopathologically there are many similarities between lichen planus and lichenoid graft-versus-host disease (GvHD). Recently, studies have shown extracorporeal photopheresis (ECP) to be effective in GvHD and chronic erosive oral lichen planus.

Patients and methods:

Four patients with erosive oral lichen planus and 13 patients with chronic cutaneous and mucosal GvHD were treated with extracorporeal photopheresis. Therapy was performed on two consecutive days (therapy cycle) every two weeks. With clinical improvement, therapy intervals were extended.

Results:

In all four patients with chronic erosive lichen planus clinical symptoms and mucosal lesions improved after seven to nine therapy cycles. A temporary worsening occurred in two patients following dental procedures. One of those patients still requires regular ECP therapy. Following nearly complete remission, two patients discontinued therapy for other reasons. One patient stopped therapy after 19 cycles of ECP therapy and has remained in complete remission for nine months.

Most patients receiving ECP during their chronic GvHD experienced a significant improvement in skin scores starting after approximately 8 therapy cycles. All patients were receiving concomitant immunosuppressive/ corticosteroid therapy and were able to reduce drug dosages during ECP therapy. Especially, gain in strength was reported by the patients. Improvement through ECP therapy strongly corresponded inversely to the degree of chronic GvHD manifestation.

Conclusions:

ECP is an effective therapeutic option for the treatment of erosive oral lichen planus, especially due to the lack of side effects occurring in other established therapies. Adjunctive topical treatment is required. Our results indicate that ECP can benefit patients with cutaneous and mucosal chronic GvHD who have failed on first- and second-line therapy. It was possible to reduce systemic immunosuppressants, especially corticosteroids, in almost all patients.

POTENTIAL PROTECTION OF SKIN BY ACUTE UVA RADIATION – FROM CELLULAR TO ANIMAL MODELS

R. M. Tyrrell
Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK

Previous studies from this laboratory have shown that ultraviolet A (UVA) irradiation of human skin fibroblasts not only strongly induces heme oxygenase 1 which catabolises heme to release free iron but also directly leads to an immediate increase in the labile iron pool as a result of ferritin degradation as well as free heme release. Both free heme and iron have been implicated in the exacerbation of inflammatory responses. Maintenance of access to these components for the synthesis of cellular proteins requires exquisite control to avoid potential cell and tissue damage. 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 these 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. Although lacking experimental verification, such a mechanistic pathway almost certainly underlies the strong anti-inflammatory activity of heme oxygenases. It therefore follows that manipulation of HO-1 regulatory pathways, including transcriptional activator proteins (e.g. Nrf2) or Bach proteins (for down-regulation) may provide a direct route to influence the protective and anti-inflammatory responses mediated by the enzyme. Heme is involved in regulation of both Nrf2 and Bach1 and recent data obtained by visualising these proteins and using specific constructs over-expressing negative regulatory proteins supports the involvement of both these proteins in UVA regulation of HO-1 activity in human skin fibroblasts . Strong expression of heme oxygenase 1 has been associated with a major anti-inflammatory response in several animal models and humans and since it is induced in most organs in the body, it has been implicated in protection against several disease states. Furthermore this induction has been shown to underlie observations in mice that have shown that the UVA waveband can modify the immune responses to UVB radiation. If more supporting data from humans is forthcoming, the possibility exists that the UVA waveband from sunlight has advantageous properties that could be harnessed for photoprotection against the suppression of cell-mediated immune function.

RMT/hct/22 Feb 05 (Put on website 22/2/05 and receive acknowledgement 23/2/05) Tyrrell/Abstracts/2005/SFRR Europe

AUTOFLUORESCENCE SPECTROSCOPY FOR CHARACTERIZATION OF HISTOLOGICAL AND BIOCHEMICAL TISSUE PROPERTIES

G. Bottiroli
IGM-CNR – Sez. Istochimica e Citometria; Dip. Biologia Animale, Università. Pavia

Most biological components involved both in functional and metabolic processes (coenzymes, flavins, lipopigments, porphyrins) and in histological tissue organization (collagen, elastin and, generally, constitutive proteins) act as endogenous fluorophores giving rise to a fluorescence emission (autofluorescence) that covers the visible range upon excitation in the UV-blue spectral region. Since autofluorescence emission is related to the nature, relative amount and spatial distribution of endogenous fluorophores, the occurrence of pathological conditions affecting histological and biochemical tissue features is expected to result in an alteration of the autofluorescence spectral properties. On this basis, autofluorescence represents an intrinsic parameter for an in situ diagnosis of tissue morfo-functional alterations through a minimally, or non-invasive, real-time technique.

Examples of autofluorescence-based diagnosis are presented, exploiting either the alteration of the tissue histological organization occurring during neoplasia growing, or the metabolic activity in relation to tissue functionality conditions.

CELLULAR AND MOLECULAR EVENTS LEADING TO THE DEVELOPMENT OF SKIN CANCER

Honnavara N. Ananthaswamy and Alessia Pacifico
The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA, and S. Gallicano Dermatological Institute, IRCCS, Rome, Italy.

Ultraviolet (UV) radiation present in sunlight causes DNA damage, inflammation, erythema, sunburn, immunosuppression, photoaging, gene mutations, and skin cancer. Upon DNA damage, the p53 tumor suppressor protein undergoes phosphorylation and translocation to the nucleus and aids in cell cycle arrest and DNA repair or causes apoptosis. Chronic UV exposure, on the other hand, overwhelms DNA repair mechanisms leading to induction of *p53* mutations. Keratinocytes carrying *p53* mutations acquire a growth advantage by virtue of their increased resistance to apoptosis. Thus resistance to cell death is a key event in photocarcinogenesis and conversely, elimination of cells containing excessive UV-induced DNA damage is a key step in protecting against skin cancer development. Apoptosis-resistant keratinocytes undergo clonal expansion that eventually leads to formation of actinic keratoses and squamous cell carcinomas. Because UV-induced *p53* mutations arise early during the development of skin cancer, discontinuation of UV treatment can still result in skin tumor development with 100% incidence, although the kinetics of tumor occurrence is delayed in the latter case. Thus, the cancer development can be delayed but not abrogated upon further avoidance of exposure to UV radiation.

In addition to mutations in the *p53* tumor suppressor gene, genetic alterations in *CDKN2A* gene leading to loss of expression of p16 ^{Ink4a} and p14^{ARF} proteins also play an important role in the development of human non-melanoma skin cancers. Our studies have shown that human basal and squamous cell carcinomas harbor mutations in the *p53* gene as well as deletions in exons 2 and 1β of the *CDKN2A* gene. While mutations in the *p53* gene are induced by UV radiation and represent tumor initiating events, mutations in the *CDKN2A* gene do not appear to be UV dependent. It is likely that these mutations arise spontaneously, perhaps during tumor progression. In summary our results indicate that mutations in *p53* and *CDKN2A* genes contribute to the initiation and progression of UV-induced skin tumors.

MOLECULAR APPROACHES TO PHOTOPROTECTION

Peter Schroeder

Increased leisure time, the growing popularity of staying outdoors and of holidays in the sun, led to the more and more important need to study the molecular and photobiological effects that ultraviolet (UV) radiation exerts on human skin. Information obtained from these studies is being used to constantly improve the quality of sunscreen preparations containing UV filters and develop new protective strategies. The development of antioxidants and active agents that can be used in combination with or in addition to UV filters to provide better photoprotection for human skin is a major task of the molecular approach in photoprotection and photoprevention.

In addition, these studies provide novel test models which allow to prove or disprove the efficacy of a given sunscreen preparation for biological endpoints that differ from the sunburn reaction and are of direct relevance for photocarcinogenesis and photoaging. The formation of the “common deletion”, a large scale deletion in mitochondrial DNA (mtDNA), in response to UV radiation is such an important relevant endpoint. Extensive studies on the topic have shown the central involvement of this 4977bp deletion in terms of photoaging and other detrimental effects in the skin. In addition to the initial damage by UV a vicious circle leads to increased common deletion levels afterwards in human skin, therefore amplifying the damage even without further UV exposure.

Based on these findings new protective strategies have to be developed to support existing approaches in photoprotection and photoprevention. In addition to decreasing the skin’s UV load by applying organic and inorganic UV filters, approaches targeting the photochemistry and photobiology of UV in biological systems have proven useful. Promising approaches include the use of creatine and supplementation with antioxidants.

SYSTEMIC PHOTOPROTECTION WITH ANTIOXIDANTS

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Sun exposure has been linked to several types of skin damage including sun burn, photoimmunosuppression, photoaging and photocarcinogenesis. Sunburn is often the first sign of excessive exposure to these damaging rays, whilst long term consequences may include photoaging and skin cancer. Currently the main method of protection against UV radiation is the use of topical sunscreens. However, there are several limiting factors with regards to the protection they provide. They need to be applied regularly (every 2 hours or immediately after swimming or strenuous activity) and getting uniform coverage over the entire body if often difficult to achieve. In view of the increasing awareness of the potentially detrimental long term side effects of chronic solar irradiation there is a general need for safe and effective photoprotectants. One likely hypothesis for the genesis of skin pathologies due to solar radiation is the increased formation of reactive oxidants and impairment of the cutaneous antioxidant system. Skin cells are well-equipped with antioxidant defences able to control the level of ROS, but in the presence of a sustained level of oxidative mediators this protective system is not always sufficient to counteract the production of ROS, determining the state of oxidative stress, which is defined as a disturbance in the prooxidant-antioxidant balances, in favour of the former, leading to potential damage. Moreover, levels of chemical antioxidants, such as glutathione, and both expression and activity of antioxidant enzymes, such as catalase or SOD, as well as melanin composition or oxidative target contents, show large individual differences, suggesting that peculiar skin characteristics can explain the existence of people highly “photosensitive”. Consequently, oral antioxidants that scavenge reactive oxidants and modulate the cellular redox status may be useful; systemic photoprotection overcomes some of the problems associated with the topical use of sunscreens. Preclinical studies amply illustrate the photoprotective properties of supplemented antioxidants, particularly RRR-alpha-tocopherol, L-ascorbate and beta-carotene. However, clinical evidence that these antioxidants prevent, retard or slow down solar skin damage is not yet convincing. The purpose of this review is to provide the reader with current information on cutaneous pathophysiology of photoxidative stress, to review the literature on antioxidant photoprotection and to discuss the caveats of the photo-oxidative stress hypothesis.

NEW STRATEGIES IN TOPICAL SUNSCREEN USE

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Sunscreens are well documented to prevent acute effects of excessive sun exposure. The sun protection factor is directly related to the ability of a sunscreen to prevent ultraviolet-induced erythema. Appreciation of the detrimental effects of ultraviolet A (UVA) has lead to the development of products able to protect against such wavelengths. Greater understanding of the ability of UVB and UVA to cause local and systemic immunosuppression has lead to broad spectrum sunscreens enabling such effects to be blocked. The addition of active agents such as antioxidants to sunscreens to further prevent photosensitivity diseases such as polymorphic light eruption has heralded development of sunscreens as active therapeutic products and not just preventing access of UV to the skin. Many new active agents are emerging some showing in vivo efficacy others still being tested in vitro. Providing issues of compliance are solved, the future for such an approach looks promising.

THE DARK AND BRIGHT SIDES OF REACTIVE OXYGEN SPECIES: HARMFUL EFFECTS OR BIOLOGICAL SIGNALLING? MOLECULAR ASPECTS OF OXIDATIVE STRESS IN PDT

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In suitable conditions and in the presence of oxygen, the interaction of light with a photosensitizable molecules may lead to the formation of chemical species (as $^1O_2$, OH°, etc) characterized by so high reactivity that they recognize intracellular targets located close to the sites of their generation. Reactive oxygen species (ROS) are frequently associated with cytotoxicity, often being described as damaging, harmful or toxic. To date, both the cell genotype and the intensity of the light-mediated oxidative stress have been considered determinant in the cell decision to die by apoptosis or necrosis. Indeed, cell death is only the macroscopically perceptible outcome of the cell resolution; indeed, more complex effects may occur that include a wide range of consequences spanning from death to unexpected proliferation. To date, accumulating evidence now suggests that ROS may act as signalling molecules for initiation and execution of molecular pathways that precisely rule the final cell response. According to such view, signalling by ROS would not appear to be random, as previously assumed, but targeted at specific metabolic and signal transduction cellular components.

Truly, few biological entities have as bad a reputation as reactive oxygen species. For many years, these small diffusible species have been regarded of as the unwanted and toxic by-products. Their endless production and harmful character has led to the generally held opinion that these species serve only detrimental functions.

Cell signalling stands on a critical paradigm: *specificity*. Classically extracellular signals are composed of growth factors, cytokines, hormones, neurotransmitters that bind to cell surface receptors. This requirement for specificity presents a real problem for those who would like to consider ROS as potentially signalling molecules, since they are so reactive that normally attack the first adjacent molecule, often over infinitesimal distances and within nanoseconds. Signalling by ROS, if does exist, has then to operate in a non conventional fashion. Consequently, *signalling specificity* cannot be based upon *molecular specificity*.

The apparent paradox in the roles of ROS as essential biomolecules in regulating cell functions and as toxic by-products of metabolism may be, at least in part, related to the differences in the concentrations of ROS produced. A classical example is provided by of NO that functions in two discrete fashions. Production of nitric oxide by macrophages and other immune-effector cells results in the high level production of NO, consistent with its role in host defence. In contrast, the nitric oxide synthase found in endothelial cells or neurons generates much much less NO when activated. Produced at this level, NO is known to function in signal transduction. This dichotomy between immune function and signal transduction is likely to be preserved for other reactive oxygen species. Both O_2^- and H_2O_2 are produced in large amounts by cells of the immune system. In contrast, other cell types, inc1uding VSM cells, chondrocytes and fibroblasts appear to produce significantly lower amounts of these molecules. Emerging evidence suggests that this ***mini*** "oxidative burst" appears to have an important role in signal transduction.

ROS as second messengers. Studies over the past few years have demonstrated that ligand stimulation on non-phagocytic cells results in an increase in intracellular reactive oxygen species. This phenomenon has been observed in a wide variety or cell types and is stimulated by a diverse collection of ligands, including cytokines, growth factors acting through Tyr kinase and G-protein-coupled receptors. The relevance of these observations have been appreciated only more recently. In vascular smooth muscle cells (VSMC) stimulation by platelet-derived growth factor (PDGF) resulted in a rapid increase in ROS which peaks within minutes after ligand stimulation and then returns to baseline with a time course similar to that of growth-factor-stimulated Tyr phosphorylation. This link between ligand-stimulated H_2O_2 production and phosphorylation greatly reinforced by the observation that exogenous H_2O_2 mimicked growth-factor-induced Tyr phosphorylation. In turn, increasing the level of the peroxide-scavenging enzyme catalase inhibited the ability of PDGF to stimulate Tyr phosphorylation. Although these results indicate that ROS may function as a second messenger system in the context of ligand stimulation, other evidences suggest that oxidative stress may also activate unique pathways.

ROS may regulate transcription. NF-kB transcription factor has been considered responsive to ROS since long time. The process initiates with an exogenous stress (ROS or else) which activates a kinase. The substrate (IkB factor) undergoes Ser phosphorylation, detachment and proteosomal degradation. The released NF-kB factor in a free form migrates into nucleus where exerts its transcriptional activity. Nonetheless, direct oxidative stress such reperfusion following hypoxia also activates NF-kB, but appears to do so through an independent pathway involving proteolysis and Tyr phosphorylation. In other systems H_2O_2 appears co-activate various PKC family members. This activation by oxidants appears to be independent of lipid cofactors and thereby differs from the classical ligand-stimulated pathway. Although less studied, even the transcriptional complex AP-1 (Fos-Jun/Jun-Jun) appears to be regulated by either by exogenous ROS or ligand-induced endogenous production of radicals.

Targets of ROS. The downstream targets of ROS have remained largely unexplored. Extracellular administration of non-lethal concentrations of H_2O_2 has been demonstrated to activate mitogen activated protein kinase (MAPK) as well as JNK, the c-Jun amino-terminal kinase a key regulator of cellular proliferation, apoptosis and tumorigenesis. The strict correlation between oxidation and MAPK cascade is further proven by the fact that ability of some ligands to activate MAPK and JNK, is inhibited in cells treated with antioxidants as N-acetylcysteine. Although the activity of the extracellular-signal-regulated kinases (ERKs) is redox sensitive, they are not direct objectives of ROS: Tyr phosphatases that have in the active site a Cys residue, have been identified as the real target. In basal conditions ROS levels are low and Tyr phosphatase activity would predominate. Ligand stimulation produces increase in ROS which transiently inactivate, by Cys oxidation, the activity of Tyr phosphatase and kinase activity becomes predominant. When ROS levels fell, phosphatase activity is restored by cellular reducing enzymes. Under such a scenario, growth-factor-stimulated ROS production would temporarily permit a burst of kinase activity through the transient inactivation of phosphatases. Recent evidence suggests that such a mechanism is common to other activators of receptor Tyr kinase activity such as radiation and alkylating agents.

The role of small GTP-binding proteins. Growth and death. One important subset of proteins involved in signal transduction is the small GTPases. Small GTPases of the Ras superfamily are monomeric guanine nucleotide-binding proteins with MW of ~25 kDa and serve as molecular switches to regulate many functions. In phagocytic cells, the small GTP-binding protein Rac2 appears to have an important role in oxidase function. Similarly, a requirement for small GTPases (Ras, Rac1) has been described for ROS generation following cytokine or growth factors. The expression of constitutively active Ras or Rac1 mutants lead to increased levels of ROS. Interesting enough while the small GTP-binding proteins Ras and Rac1 may contribute to ROS production they are themselves target of oxygen radicals as demonstrated by the presence of a ROS-sensitive Cys in their sequence. As cell growth is concerned it appears that low levels of ROS in some cells stimulate proliferation and expression of growth-related gene products. Conversely, ROS may mediate apoptotic pathways. Recent studies observed that stimulation with Fas ligand resulted in apoptosis through superoxide generation; such process was inhibited by expression of a dominant-negative *ras* gene. Other studies reported hat ROS may mediate p53-dependent apoptosis and many other genes that regulate the redox state of the cell.

As long as PDT is concerned, it is clear that the ROS are generated with the purpose of destroying the host malignant cells. However, we should be aware that because the subcellular localization of the photosensitizer, because the scant photoactivation of cells just rubbed by light at the border of the light spot or below the tumor, the production of ROS is low and may trigger unwanted signalling.

PREVENTION OF PHOTODERMATOSES: THE ROLE OF OXIDATIVE STRESS

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Polymorphic light eruption (PLE) is the most common photodermatosis. While its etiology still remains elusive, pathogenesis seems to involve UVA-induced oxidative stress and subsequent deregulation of antioxidant immune responses. Only few and often ineffective prophylactic and therapeutic measures exist to date.

One of the key factors appears to be the generation of reactive oxygen species (ROS) by UVA radiation and subsequent up-regulation of pro-inflammatory and immune modulating cytokines and adhesion molecules. Several studies have shown that oxidative stress in vivo can lead to alteration of proteins, lipid peroxidation and oxidative DNA damage. Subsequently, this may cause dysfunction of cellular structures, induction of heat shock proteins, as well as formation of neo-antigens and/or initiation of immune reactions. UVA irradiation induces the expression of pro-inflammatory genes in human keratinocytes and may thus play a major role in the pathogenesis of photoinduced skin diseases, such as PLE. Prophylactic and therapeutic measures for PLE are often not very effective and usually sunscreens do not provide a complete protection against UVA irradiation. Combination of potent antioxidants with a broad spectrum highly UVA protective sunscreen is far more effective in preventing PLE than sunscreen alone. We therefore propose the assumption of systemic antioxidants and the topical application of an efficient UV filter system, with a strong absorption in the UVA range, as an effective mean to prevent and counteract the oxidative stress in the skin. By influencing this response in an early phase, the homeostasis of the endogenous redox system in the skin can be maintained and the elicitation of clinical symptoms of PLE can be prevented.

PHOTOREJUVENATION: WHAT'S NEW

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Heat-induced damage by intense light has been hypothesized to result in the activation of dermal fibroblast via the process of tissue healing response during wound repair. Reports have been issued on improvement in the appearance of fine lines and wrinkles when using intense light near and mid-infrared band.

A multicenter and plurinational study was set up to evaluate the efficacy and safety of a High Impact Infrared pulsed light system of particular characteristics as a nonablative treatment for facial rhytides and skin remodeling. A controlled pulsed light device Novaplus ® by Ultramed (Geneva, Switzerland), which emits pulses in the broadband spectrum of light from 900nm to 1500 nm (with a skin contact cooling at 5 C.) was used to treat periorbital wrinkles. Treatment was done with a train of pulses of 2000 ms in total, with fluences of around 30J/cm^2. Pulses were stacked two to three times on the face except on bony areas where only one pulse was delivered without any type of anesthesia.

The 50 patients treated had skin phototypes I to VI, Fitzpatrick photodamage scores 3 to 9 and had had no previous facial aesthetic treatments. All patients received four treatment sessions at 2 week intervals. Areas of treatment were photographed with standardized settings, and 3D images for computer analysis through video microtopography was implemented for objective assessment. Subjects were asked to rate the level of pain on a visual analogue scale.

Results will be presented as per the evaluation done by patients via several questionnaires, and objective assessment by a blinded specialist and computer analysis based on randomized photographs to grade wrinkle severity. Also, subjective analysis of results for fine wrinkles, roughness, coarseness and skin toning before and at three months after the last treatment session by all patients provided interesting results.

MODERN TREATMENT OF SOLAR URTICARIA

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Idiopathic solar urticaria (SU) is a rare but severely disabling, sometimes even life-threatening photosensitive disease. Although a variety of treatment modalities have been tried, their effectiveness is disappointing in most cases. This is particularly the case with drug therapies, such as antihistamines, chloroquin and immunosupressive drugs. The most effective therapy for SU still appears to be the induction of tolerance by applying graded subthreshold UV doses (UV hardening). However, this strategy is laborious, time consuming and of prolonged duration until protection is achieved. We have therefore developed a UV rush hardening treatment regimen. This UV rush hardening has been successfully introduced for the treatment of three patients with spontaneously occuring severe primary solar urticaria. UVA exposures were started with half of the minimal whealing dose, increased by 30% and given multiple times per day. Within only 3 days a UVA dose of 10 J/m^2 was achieved and well tolerated by all patients. Subsequent photoprovocation by applying 50 J/m^2 UVA was negative. Tolerance maintaining exposures were given only once a week. In all 3 patients no urticarial lesions developed despite of normal outdoor activities. Taken together, UVA *rush* hardening appears to be a safe and effective regimen for the treatment of idiopathic SU, which provides protection within 3 days.

PHOTOSENSITIVITY AND PHOTOTESTING IN CUTANEOUS LE

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Photosensitivity in lupus erythematosus (LE) shows a strong association to disease manifestation suggesting that abnormal reactivity to ultraviolet (UV) light is one important factor in the pathogenesis of this disease. A standardized test protocol has been developed to evaluate photosensitivity in patients with cutaneous manifestations of this disease including that defined areas of uninvolved skin on the upper back or extensor aspects of the arms are irradiated with single doses of 60-100 J/cm^2 UVA and/or 1.5 MED-UVB, respectively, daily for 3 consecutive days. The evaluation follows after 24, 48, and 72 hours and weekly up to 3 weeks after the last iradiation. In 2001, we reported on our experience of provocative phototesting in 400 patients with various subtypes of LE and demonstrated that skin lesions can be induced by UV irradiation in more than 50% of the patients. Interestingly, the onset of positive phototest reactions in LE is significantly slower than in other UV-induced dermatoses, such as polymorphous light eruption. Furthermore, we recently showed that broadband sunscreens are able to suppress the induction of skin lesions on UV irradiation in patients with LE using this standardized phototesting procedure. Therefore, consequent protection against UV light is of significant value for the course and prognosis of this disease. Moreover, this testing regimen has received much attention because the capacity of UVA and UVB irradiation to reproduce LE skin lesions is an ideal model for several experimental approaches, which allows the study of inflammatory and immunologic events that take place prior to lesion formation. Further elucidation of the various factors that contribute to the UV initiation and perpetuation of autoimmune responses may lead to future developments of more specific pharmaceuticals beyond UV filters to prevent induction and exacerbation of LE and to counteract the detrimental effects of UV irradiation on the disease.

PHOTODYNAMIC THERAPY OF NON-MELANOMA SKIN CANCER: UPDATE 2006

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Photodynamic therapy (PDT) with 5-aminolevulinic acid (ALA) and its derivative methylaminolevulinate (MAL) have been investigated widely over the past two decades and MAL- PDT is now approved by the European regulatory authorities for the treatment of actinic keratosis and basal cell carcinoma where it proved a simple, safe and effective therapeutic modality with sevsral advantages in comparison to standard surgical procedures: it avoids bleeding, is well tolerated by patients and improves the cosmetic results. In addition, ALA or MAL PDT proved a promising treatment option for other skin tumours (Bowen's disease and cutaneous T cell lymphoma), several inflammatory diseases (e.g. psoriasis, acne vulgaris, hypertrichosis, LED, sarcoidosis and annular granuloma), and skin infections (e.g. warts, HPV-induced condylomata and mycosis). Photorejuvenation is another promising application.

In addition several new sensitizers, e.g. chlorins, porphycenes, phthalocyanines, and hypericin are investigated in pilot studies as well as in phase II and III studies for non-melanoma skin cancers and Kaposi's Sarcoma, bacterial and mycotic skin infections as well as psoriasis. All these dyes are used after systemic parenteral delivery and some of them are available for topical use too. All these new "second generation" photosensitizers are characterized by very high extinction coefficient and singlet oxygen quantum yield. In addition, they have a high absorbance in the far red or near infrared wavebands allowing the treatment of thick lesions infiltrating the dermis. Finally they have no dark toxicity and, in contrast to "first generation" sens, e.g. hematoporphyrin derivatives and Photofrin®, they are characterized by short-lasting generalized skin photosensitivity.

SYNTHESIS AND DEVELOPMENT OF NEW PHTHALOCYANINE DERIVATIVES FOR ANTIBACTERIAL PHOTODYNAMIC THERAPY

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The appearance of resistance to the antibiotic treatment for many pathogens is one of the most serious problem in the realm of microbial infections. The extensive and sometime inappropriate use of antibiotics has lead to the emergence and spread of this problem worldwide. The resistance once localized and fostered in the hospitals, are spreading to surroundings microorganisms communities due to sharing of resistance genes. About 25 % of annual deaths worldwide are estimated to be related directly to infectious diseases not including the deaths occurring as a consequence of past infections, 20% of infections in hospitals involve multidrug-resistant bacteria, while the pipeline of classic antibiotic drugs development is running dry.
New drugs and therapeutic approaches based on different strategies are thus urgently needed in this field and photodynamic therapy (PDT) could represent a suitable alternative especially for the treatment of localized infectious diseases, including those caused from drug and multidrug-resistant microorganism.

Systematic synthetic modifications on phthalocyanines having interesting characteristics as antimicrobial photosensitizers and detailed structure activities relationships studies have been extensively performed in our laboratories. These have allowed the identification of a number of Zn(II)-phthalocyanines with the following characteristics:

1. Broad spectrum activity against several classes of microbial pathogens, including Gram + and Gram- bacteria, fungi, yeasts and parasites. By using standard phototerapeutic protocols 4-5 log decrease in the microbial population can be easily obtained at concentration in the 0.05-1 μM range and irradiation times of few minutes.
2. Overall hydrophilic/hydrophobic balance of the phthalocyanine macrocycle modified by synthesis, in order to obtain molecules able to specifically inactivate Gram + or Gram- bacteria as well as yeasts.
3. As phthalocyanines cause microbial cell death by a multi targeted process mainly involving the cytoplasmic membrane, the photoinactivation process does not allow for the selection of PDT resistant species.
4. Due to the different mechanism of action compared to antibiotics, the photoinactivation of microorganism with phthalocyanines is very similar for both wild and antibiotic resistant strains.
5. The photodynamic process of inactivation is not affected by the previous treatment of the microbial infection with antibiotics, thus combination therapies are possible
6. As the photodynamic effect induced by phthalocyanines predominantly act at the level of the plasma membrane, the risk of inducing mutagenic effects is minimized and no genotoxicity has been found for the phthalocyanine derivatives developed so far.

Microorganisms inactivation by using *in vitro* validated PDT protocols have been transferred to treatment of *in vivo* in animal models as well as to the treatment of spontaneous cutaneous infections. Dogs with infected chronicized lesions not longer responsive to the antibiotic treatment have been successfully cured by using the phthalocyanines developed and the PDT approach. The results obtained so far may anticipate the favourable applications of antibacterial PDT in the treatment of wound infections, oral and vaginal candidosis, and superinfections.

OXIDATIVE DAMAGE IN VITILIGO

Bernhard Ortel

The physiopathology of vitiligo is far from being completely understood. Several concepts have been put forward but none has satisfied all aspects of the disease process. There is evidence that genetic factors, autoimmune mechanisms, and oxidative damage may well all play a role in the manifestation of this acquired loss of epidermal pigment. The review will describe evidence of oxidative agents and pathways that play an important role in the development of vitiligo. The investigation of chemical leukoderma and chemically induced vitiligo may be an important segue to understanding pathogenetic mechanisms. The increased susceptibility of melanocytes to oxidative damage is associated with altered defense mechanisms against oxidants in vitiligo patients. The role of anti-oxidants in the prevention and treatment of vitiligo will be discussed.

APPLICATIONS OF PHOTODYNAMIC THERAPY IN THE TREATMENT OF MICROBIAL INFECTIONS

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Photodynamic therapy (PDT) is coming of age as an efficient alternative treatment for microbial infections, a problem which is presently aggravated by the increasingly widespread diffusion of antibiotic-resistant microbial strains. In particular, the use of red light-absorbing photosensitisers as photodynamic antimicrobial agents is characterized by various favourable features, including: (a) the broad spectrum of antimicrobial action of selected phenothiazines, porphyrins, and phthalocyanines, which promote the photosensitised inactivation of Gram(+) and Gram(-) bacteria, fungi, mycoplasma, and parasites in both the vegetative and cystic stage by using one phototherapeutic protocol and mild irradiation conditions; (b) porphyrins/phthalocyanines display no appreciable toxicity in the dark at photochemically active doses; (c) a therapeutic window can be identified which allows an extensive (> 4 log) decrease in pathogen survival with no detectable damage to the host tissue; (d) microbial cell death is primarily a consequence of membrane photodamage through a typically multi-target process, which minimizes the risk of both the onset of mutagenic processes and the selection of photoresistant cells; (e) such photosensitisers act with essentially identical efficiency against both wild and antibiotic-resistant strains, whereas no selection of photoresistant microbial pathogens has been observed; (f) a combination between antibiotic-based and photodynamic therapy is possible. At present, antimicrobial PDT appears to be especially convenient for the treatment of localized infections, such as oral candidosis, acne, periodontitis or chronic wounds.

WORKSHOP ON PHOTODYNAMIC THERAPY

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The Workshop on Photodynamic Therapy (PDT), the "new frontier" of the photodermatological treatment, aims to explain the use of this therapeutic technique, by a teorical-practical educational course, that starts from the European registered indications (actinic keratosis, superficial and nodular basal cell carcinoma) with photosensitizers for local use, and especially those already commercially available and registered, and leads to the future interesting applications with methyl-aminolevulinate and other photosensitizers. On the basis of data of the literature and the authors' experience we'll analyse all the basic phases (treatment protocol) of treatment from the preliminary visit and patients' selection and indication to the preparation of the lesion to be treated, the treatment modality, the post-treatment and side effects. Therefore the Workshop will give some suggestion about response evaluation and legal items especially on information sheet for patients and informed consent.

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LOW DOSE UVA1 PHOTOTHERAPY IS EFFECTIVE IN THE TREATMENT OF UVB-INDUCED CUTANEOUS LESIONS OF LUPUS ERYTHEMATOSUS

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Unlike sunlight and artificial ultraviolet B (UVB) (280-320 nm) radiation, ultraviolet-A1 (UVA1) (340-400 nm) proved effective in the treatment of LE skin lesions. The aim of the present study was to assess the efficacy and tolerability of low-dose UVA1 phototherapy for the treatment of skin lesions of 1 patient with subacute cutaneous lupus erythematosus (SCLE) and 2 patients with systemic LE (SLE). After provocative UVA and UVB phototesting low dose (20 J/cm^2) UVA1 exposures were delivered 3 times weekly until complete remission of cutaneous lesions or until partial improvement was achieved without further amelioration despite another week of treatment. The patient with SCLE showed a complete remission of skin lesions without recurrences at follow-up (4 years). Contrasting results were obtained in the two patients with SLE: a patient had a partial remission with persistence of less of 25% of the lesions whereas the other one had an exacerbation of skin lesions. The therapeutic activity of UVA1 phototherapy seems to be related to a complex network of immunomodulatory activities, including induction of apoptosis of T-cells present in lesional skin, promotion of DNA-repair, decrease of IL-12, TNF-α and IFN-γ. Further controlled and randomized studies are needed to determine criteria of selection of patients, efficacy and long term adverse effects.

MAL-PDT OF DISCOID LUPUS ERYTHEMATOSUS

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Photodynamic therapy with methylaminolevulinate (MAL) cream (Metvix®, Galderma, F) has a strong modulatory activity of immunocompetent cells located in both epidermis and dermis. Discoid lupus erythematosus (DLE) is a localized skin disease characterized by an intense dermo-epidermal lymphomonocytic infiltrate. Standard treatment approaches are sometimes uneffective and/ or are followed by systemic and local toxicity. We treated two patients with DLE of the face unresponsive to standard and experimental topical, intralesional and systemic therapies.

We treated two patients with histology- proven DLE of the face with MAL-PDT (once weekly for 4 weeks). Treatments were well tolerated and at the end of the treatment cycle, lesions showed a significant improvement. Relapses occurred after 1 and 3 months respectively and were amenable to a second treatment cycle.

DERMATITE POLIMORFA SOLARE O LUCITE ESTIVALE BENIGNA, QUALE DIFFERENZA. RISULTATI DI UNO STUDIO ITALIANO

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La dermatite polimorfa solare è la fotodermatite idiopatica più frequente in assoluto. Da tempo alcuni autori francesi e italiani hanno distinto una forma più lieve chiamata lucite estivale benigna (LEB) e la dermatite polimorfa solare (DPS) vera e propria. Sono state indicate alcune caratteristiche differenziali tra le due forme, ad esempio la sede e il sesso più frequentemente colpito, la morfologia delle lesioni, il tempo di latenza delle manifestazioni, le recidive annuali o la positività al fototest. Il gruppo GIFDE ha raccolto 297 cartelle anamnestiche di DPS da centri dermatologici di diverse città italiane (Genova, Napoli, Brescia, Verona) individuando le caratteristiche utili alla definizione delle due forme cliniche. Vengono presentati e discussi i risultati.

SERUM LIPIDS, LIPOPROTEINS, AUTOANTIBODIES AGAINST OXIDIZED LDL AND TOTAL PEROXIDE LEVELS IN RELATION TO PLASMA IL-18 LEVEL AND PERIPHERAL BLOOD DENDRITIC CELL POPULATIONS IN MEN WITH PSORIASIS

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Background. Findings from recent large-scale epidemiological studies point to significantly increased risk of cardiovascular diseases (CVD) in patients with psoriasis (J Invest Dermatol 2006;126;4:47). However, the mechanisms responsible for this relationships are still not clear. Since psoriasis is an immune-mediated disease we undertook to investigate associations between known potential risk factors for CVD and selected indices of the immune response in psoriasis to shed more light on the relationships between CVD and this skin disease.

Material and methods. Thirty-four men with psoriasis and 26 healthy men took part in the study. Patients and controls were particularly matched for BMI, age, and alcohol and tobacco use. Serum lipid, lipoproteins, autoantibodies against oxidized LDL (AuAb-oxLDL) and total peroxide concentration patterns were measured as the potential factors known to play a role in CVD. Additionally, peripheral blood dendritic cells (DC) and plasma IL-18 levels were measured as indices of the immune response in psoriasis. Standard enzymatic-colorimetric techniques were used to assess the serum concentration of lipid parameters (BioMěrieux, France) and total peroxides (OxyStat BIOMEDICA GRUPPE Austria). The flow cytometry method was utilized to measure blood DC subpopulations. Turbidimetric techniques were applied to assess ApoAI, ApoB (DadeBehring Marburg GmbH-Turbiquant Apolipoprotein A-I, Apolipoprotein B) and Lp(a) (Human) concentrations. The presence of IL-18 (MBL, Japan) and AuAb-oxLDL LDL (OLAB- BIOMEDICA GRUPPE Austria) was detected with the Elisa kits. Patients with psoriasis were also assessed on Psoriasis Area and Severity Index (PASI), percentage of affected skin, duration of the disease and duration of the current relapse. Differences between patients and controls were calculated on the assessed parameters, and the correlation coefficients between potential risk factors for CVD and indices of the immune response and clinical characteristics of the disease were assessed in the group of patients.

Results. We found no differences between psoriasis patients and healthy controls in the total serum cholesterol level. However, patients with psoriasis had significantly lower levels of HDL cholesterol and significantly increased levels of lipoprotein A-I, AuAb-oxLDL and total peroxide concentration in comparison to the control group. Blood DC subset BDCA2 count was significantly decreased and plasma IL-18 level was significantly increased in patients with psoriasis as compared to healthy controls. In the group of patients, the assessed potential risk factors for CVD were found to correlate significantly both with indices of the immune response in psoriasis, such as DC counts and IL-18 level, and with clinical characteristics of psoriasis, such as the PASI score, percentage of lesional skin and duration of the disease.

Conclusions. Our results suggest a disturbed pattern of cholesterol fractions, lipoprotein A-I, AuAb-oxLDL and the total peroxide concentration in patients with psoriasis. Potential risk factors for CVD were shown to be associated with both immunological parameters and clinical features of psoriasis, which suggests that psoriatic processes and can be pathogenetically linked to factors playing a role in CVD.

PHOTOTHERAPY WITH RED LIGHT FOR THE TREATMENT OF MODERATE ACNE VULGARIS.

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During the last 20 years, a growing number of topical, systemic and physical treatments for acne vulgaris have been investigated.

Recently, the effects of various phototherapeutic protocols with visible (blue and red) lights have been investigated with open uncontrolled trials. Mechanisms of action are unknown although photodynamic damage of P. Acnes seems the most important biological effect underlying the therapeutic activity.

In the present study, 15 women suffering from comedonic, popular and pustolar acne vulgaris of the face were treated. The whole face was exposed to 20 J/cm2 of red light twice a week for 4 weeks. Treatments were well tolerated with only a mild transitory discomfort.

All patients improved with a sustained efficacy at a 3 months' follow-up.

Present findings show that phototherapy with red light might be an alternative treatment option for moderate acne vulgaris in patients who do not respond to conventional drugs.

TREATMENT OF PHOTODAMAGED FACIAL AND SCALP SKIN USING MAL-PDT

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Efficacy and tolerability of photodynamic therapy (PDT) with aminolevulinic acid (ALA) in the treatment of photodamaged skin, so called photorejuvenation, have been reported in a few recent studies. However, results are hardly comparable because there were evaluated only on a clinical basis and the treatment protocols, e.g. formulation and concentration of the cream, application time, spectrum and dose of the activating light, number and frequency of treatments, varied widely.

In the present study, 20 patients with pronounced photodamage and actinic keratoses (AK) of the face and scalp were treated with two monthly treatments with a proprietary preparation containing 160 mg/gr of methylaminolevulinate (MAL) (Metvix®, Galderma, F) according to the standard treatment protocol that is approved by the European regulatory authorities for the treatment of AK. In brief, Metvix® was applied under occlusion for 3 hours before exposure to 37 J/cm^2 of red light that was delivered by a LED source (Aklilite® CL 128, Photocure, N).

Improvement of different clinical signs evaluated separately according to a clinical scale and, in addition, photodamaged skin has been evaluated with high-resolution 20 MHz B-Mode ultrasound scanner before and after the last treatment.

Treatments were well tolerated with only a mild and transitory pain and burning. Soon after the treatment, the skin showed diffuse erythema and oedema that were more pronounced with crusting in the AK areas. These changes resolved spontaneously without scarring or pigmentary alterations within 5 days.

MAL-PDT could represent a novel, effective and well- tolerated non-invasive treatment of photodamaged skin.

LIVER AUTOFLUORESCENCE IN EU- AND IPERTHYROID RATS

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PURPOSE: to investigate liver autofluorescence properties under Thyroid hormones (THs) induced metabolic alteration. Liver autofluorescence results from the contribution of several endogenous fluorophores, in relation with the organ complex biochemical composition, due to its metabolic and detoxificating roles. NAD(P)H and flavins coenzymes fluorescence is strictly dependent on cells redox state, in close relationship with metabolic activity. THs are critical for energy homeostasis regulation. Their increase enhances oxygen consumption and heat production. In hepatocytes THs induce short-term effect (alteration of cytochrome-oxydase-complex kinetics properties, within minutes), and long-term effects (up-regulation of nuclear encoded respiratory genes; increase in glycerol phosphate dehydrogenase shuttle –GPDH, ever since few hours).
METHODS: L-thyroxine (30mg/100g b.w.) was administered i.p. to male Wistar rats for 7 consecutive days. On the eighth day Euthyroid and thyroxine-pretreated rat livers were exposed in vivo to 20-minglobal ischemia, then to 30 min reperfusion. In situ spectrofluorometric analysis was performed via single optical fibre probe (PMA 11 Hamamatsu OMA system, 366 nm exc.).
RESULTS: Under unperturbed blood circulation no appreciable differences occurred between autofluorescence properties from livers of eu- and iperthyroid rats. In euthyroid rats warm ischemia induced a transient emission decrease, followed by an increase and stabilization at values higher than t0 ones (+15%). Circulation restoring resulted in a decrease of autofluorescence signal (-35%). Hyperthyroid in comparison with euthyroid rats exhibited a similar behaviour, but with significantly higher signals, and faster and slower answers to ischemia and reperfusion, respectively. Spectral fitting analysis evidenced that flavins and free NAD(P)H were the fluorophores responsible for the greater differences between eu- and hyperthyroid rats, for both for their relative contribution to the whole emission spectrum and for their absolute values.
CONCLUSIONS: Data obtained indicate a larger production and accumulation of free NAD(P)H in hyper- than in euthyroid rats. This is in full agreement with the increase in GPDH shuttle activity, enhancing the reducing-equivalents flow capacity of respiratory chain and the reoxidation of NADH and ATP production via glycolysis, as the hepatocytes answer to THs.
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DISINFECTION OF WATER FROM TROUT FARMING POOLS AFFECTED BY SAPROLEGNIOSIS THROUGH THE COMBINED ACTION OF PORPHYRINS AND VISIBLE LIGHT

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Saprolegniosis is an ubiquitous fish mycosis. It frequently develops in fresh water farming and, therefore, it induces considerable damage to both egg production, which reduces hatching, and adult fishes, which causes morbidity and death of infected subjects. To combat saprolegniosis formalin is often used as an antimycotic agent, even though without regulatory authorization, because of its possible carcinogenic and teratogenic properties. Therefore, it appears necessary to replace this disinfectant by a new product that combines efficacy and safety for operators and consumers. Porphyrins, both in the dark and activated by visible or sun light, appear to be suitable for replacing formalin, since they represent an efficient antimycotic agent, not dangerous for human health and with a low environmental impact. In this work photosensitisation by porphyrins and their derivatives is applied for the first time to control fungal population in a modern plant for fish farming. In particular, we focused our attention on the treatment of Saprolegnia-induced infections. At first we performed porphyrin uptake and photosensitization studies on Saprolegnia; on the basis of these results, we applied porphyrin photosensitization on trout eggs and adult fishes. Results show that porphyrins are significantly accumulated by fungal cells and induce photosensitivity. The efficacy of porphyrin action on trout eggs is similar to that exhibited by the present disinfectants, while the treatment of adult trouts has a preventive effect toward development of infections, and has an efficient curative action on infected fishes. We are planning further investigations as regards porphyrin localization and cell death mechanism by fluorescence and trasmission electron microscopy.

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RAPID-ONSET EFFECT OF VISIBLE LIGHT IRRADIATION ON THE OXYGEN CONSUMPTION RATE IN CULTURED CELLS

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A growing number of studies have reported on the biological effects of monochromatic red or near-infrared light (Karu, 1999). The results of these studies has been related to potential phototherapeutic effects in several pathological conditions (mainly dermatological, but also retinic alterations). It has been hypothesized that these effects are mediated by specific photoacceptor biomolecules, the most probable candidate being the mitochondrial enzyme cytochrome *c* oxidase (CoOX) (Karu *et al.,* 2004). Around these studies there has been a live debate in the scientific community, but the obvious interest for their possible biomedical relevance is shown by the fact that irradiation devices with red and near infrared (NIR) LED have been designed and produced as experimental prototypes, currently used by NASA (Whelan *et al.,* 2003).

We investigated the effects of visible-to-near-infrared (Vis-NIR) irradiation on the *in vitro* activity of a purified and stabilized form of cytochrome *c* oxidase and on the *in vivo* activity of the same enzyme in cultured cells. The enzyme activity assay *during* Vis-NIR irradiation has been carried out using a Clark electrode to measure oxygen consumption (under conditions of steady-state reduction of cytochrome *c* by an ascorbate/TMPD mixture). Our results show that the activity of purified CcOX is unaffected by irradiation with any frequencies of Vis-NIR light delivered from a Xenon lamp or by irradiation with He-Ne laser. The same kind of experiments have been carried out on eukaryotic cells of different taxa: cultured mammalian cells, the ciliate *Tetrahymena thermophila* and the yeast *Saccharomyces cerevisiae*, always under conditions that allowed sample irradiation *during* activity assay. In these measurements the irradiation with red-NIR light had no immediate effect on oxygen consumption (which may be ascribed to CcOX under our experimental conditions). On the other hand, irradiation of *T. thermophila* cells with visible light induced a sudden increase in oxygen consumption rate, which returned to its basal value in few seconds after the light was turned off. Most of this effect was due to blue light (420±30 nm), while red light (> 600 nm) produced a lower effect than blue light. Preliminary experiments on cultured mammalian cells and on aerobically grown yeast cells have shown that in these systems too the oxygen consumption rate is increased by irradiating the cells with visible light. It is most interesting that the irradiation on anaerobically grown yeast cells has no relevant effect. These results seem to confirm that CcOX is the specific photoacceptor involved in these phenomena.

To the best of our knowledge, this kind of irradiation effect in such biological systems has not been previously reported. Although further experimental results are needed to fully appreciate its biological meaning, it could suggest an underlying mechanism of photomodulation of the respiratory rate in eukaryotic cells.

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PHOTOTOXICITY OF SOME ANTIMALARIAL DRUGS

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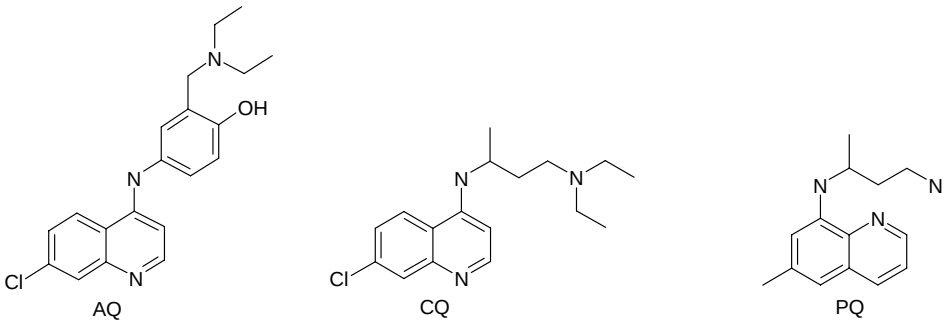
Many antimalarial drugs derived by quinoline show phototoxic properties that cause adverse effects in the skin and in the eyes. The photoactivity of three antimalarial drugs, Amodiaquine (AQ), Chloroquine (CQ) and Primaquine (PQ), was investigated through a series of *in vitro* tests to understand the action mechanism and the photoinduced effects on the main cellular targets.



A certain phototoxicity was observed for all the drugs on human keratynocytes (NCTC-2544) and mouse fibroblasts (3T3) cell lines but the most phototoxic drug was PQ. A cellular viability test was carried out in presence of some scavengers during irradiation to identify of the reactive oxygen species involved in inducing phototoxicity. Changes in cellular cycles of NCTC-2544, irradiated in presence of the drugs, were evaluated through flow cytofluorimetry with the purpose of clarifying the way of cellular death. With the same purpose, a biparametric cytofluorimetric analysis was performed using propidium iodide and AnnexinV-FITC, which stain DNA and phosphatidylserine residues respectively. The following step was the valuation of the involvement of cellular organelles in cellular death. We studied the possible mitochondrial dysfunction through flow cytofluorimetric trials: the alteration of mitochondrial membrane potential using JC-1 probe and the production of ROS as a consequence of respiratory chain disruption with probes such as Hydroethydine (HE) and 2,7-dichlorofluorescein (DCF). Cells were assessed for lysosomial stability using the Acridine Orange (AO) uptake method. We observed an involvement of mitochondria but not of lysosomes in inducing cell death. Finally we also analysed the interaction of the drugs with DNA and the possible photodamage on proteins and lipid peroxidation.

UVA-ACTIVATED FUROCOUMARINS INDUCE ACCUMULATION OF γ-GLOBIN mRNA IN HUMAN ERYTHROID CELLS

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The search for potential therapeutic agents in haematological disease including β- thalassemia and sickle cell anemia (SCA), focuses on the pharmacologically mediated regulation of the expression of human γ-globin genes, leading to the production of fetal haemoglobin (HbF). In this context many studies have been started to find natural or synthetic compounds capable of augmenting HbF levels in humans. In particular emphasis has been given to DNA-binding agents which appear of special interest and among the molecules able to interact with DNA, psoralen and related compounds could be relevant. In this study we have analysed several linear and angular psoralen after UVA irradiation, employing two experimental cell systems such as the human erythroleukemia K562 cell line and the two phase-liquid culture of human erythroid progenitors isolated from normal donors for further analysis on the most active compounds. The obtained results showed that psoralen, angelicin and several structurally related compounds (trimethylangelicin, 5-methoxypsoralen, 4-methylpsoralen and 5'-methylpsoralen) are powerful inducers of erythroid differentiation evaluated by benzidine staining and γ-globin mRNA accumulation both in K562 cells and in human erythroid precursors. Interestingly the activity of some compounds such as angelicin was found higher than that displayed by hydroxyurea, a drug commonly used as HbF inducer in β- thalassemia and SCA patients.

These results could have a practical relevance because pharmacologically mediated regulation of the expression of human γ-globin genes leading to increased HbF production, is considered a potential therapeutic approach in haematological disorders, including β-thalassemia and SCA.

INTERACTION BETWEEN CISPLATIN AND PHOTODYNAMIC THERAPY WITH PHOTOFRIN IN ESOPHAGEAL CANCER CELLS

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Traditional cancer therapies, such as chemotherapy, involve a delicate balance between destroying diseased tissue and sparing surrounding normal healthy cells. These conventional treatments cause the loss of normal cell function as a result of having relatively indiscriminate cytotoxic properties. Consequently, the development of treatment protocols that display more selectivity for disease tissue is very important. Photodynamic therapy (PDT) is recognized as an effective anticancer procedure for various types of solid tumor, but its combination with other established therapeutic treatments has not been evaluated in details.

The aim of this work was to evaluate the possibility of the additive/synergistic effects induced by the combination of chemotherapy and PDT in *in vitro* cancer cells.

The esophageal squamous carcinoma cells (KYSE-510) were dark-incubated for 16 hrs with 2.5 μg/ml Photofrin, as the photodynamic agent, and irradiated with increasing doses of red light to define viability curve as a function of the light dose. PDT was performed by irradiating Photofrin-preloaded KYSE-510 cells with red light emitted from a halogen lamp equipped with a bandpass filter. The dose-response curve was also established after cell exposure to increasing doses of cisplatin, a chemotherapic currently used in standard protocols for the treatment of esophageal cancer.

Viability of the cells was measured by trypan blue exclusion assay 24 hrs after treatment.

The data were used for selecting appropriate experimental conditions for subsequent experiments in which the two treatments were combined. In particular, in the combination experiments the fluence rate was 1.8 J/cm^2 since at this dose PDT monotherapy reduced KYSE-510 cell viability to ~50% that of unirradiated cells.

The combination of cisplatin and PDT did not significantly increased the death of KYSE-510 cells, as compared to single treatments.

In order to clarify the reasons of the observed effects, the cell cycle profiles and the mechanisms of apoptosis, induced by the single and the combined treatments, are under investigation.

EFFECTS OF VARIOUS EXPERIMENTAL PARAMETERS ON THE PHOTOINACTIVATION OF *S.aureus* SENSITIZED BY XF-73

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The increasing emergence of antibiotic resistance among pathogenic bacteria led to the development of alternative methods for the treatment of infectious diseases. Photodynamic Therapy (PDT) with cationic photosensitizers appears to be a very promising approach for the treatment of localized microbial infections. This study evaluated the influence of several experimental parameters of the antibacterial activity of XF-73, a dicationic porphyrin, exhibiting a high photosensitising activity against several strains of both Gram-positive and Gram-negative bacteria. The experimental parameters analysed were: light fluence rate, incubation time, number of cell washings before irradiation, concentration of ions in the buffer used for resuspending cells.

S.aureus cells in the stationary phase of growth (10^8 cells/ml) were incubated with 2.5-10 nM XF-73, exposed to blue light (13.7 J/cm^2) without washings or after three washings. The cell survival was measured by the CFU assay.

Our results demonstrate that the efficiency of *S.aureus* inactivation does not increase significantly by prolonging the time of incubation before irradiation from 2 to 20 minutes.

The cells irradiated with a light dose of 13.7 J/cm^2 delivered at fluence rates of 15 and 35 mW/cm^2 show slightly different survival of respectively 1.4% and 0.3%.

The efficiency of photoinactivation is slightly higher when the cells are exposed to light without removal of the free porphyrin in comparison to the cells subjected to three washes before irradiation.

We have also shown that the concentration of cations Na^+ and Ca^{2+} does not affect negatively the photosensitising activity of XF-73 toward *S.aureus*.

These data suggest that the antibacterial activity of XF-73 is not strongly affected by the experimental conditions and is potentially useful for the treatment of superficial and localized bacteria infections with PDT.

IN VITRO AND *EX VIVO* PHOTOSTABILITY STUDIES ON FLUMETHASONE AND FLUOCINOLONE ACETONIDE

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Flumethasone and Fluocinolone acetonide are synthetic corticosteroids used topically for the short-term treatment of various inflammatory skin diseases such as eczema, eye, nose, and ear infections, stomatitis and psoriasis.

As other 11β-hydroxy corticosteroids, they have shown to be sensitive to light. Previous studies have demonstrated their reactivity under UV light (UVB>UVA) both in organic and aqueous solvents and their main photoproducts have been isolated and characterized. For this high instability to UV irradiation, these drugs may lose their therapeutic activity. Indeed, their packaging instructions always indicate to protect them from light during storage. Moreover, the same problem may arise when patients are exposed to solar radiation. In order to verify the instability of these molecules when applied on the skin under UV light, the photodegradation of the two drugs is studied both *in vitro* (aqueous solution and commercial formulation) and in the pig skin; this *ex vivo* model, very close to the human skin, was used to study the fate of drugs in this organ when exposed to light.

Both the glucocorticosteroids applied on the pig skin showed high photoinstability under UVB irradiation (up to 40 % for Flumethasone and to 30 % for Fluocinolone acetonide, under 10J/cm^2 of UVB). The photoproducts formed in the skin were the same found *in vitro*, except for the hydroperoxide of Fluocinolone. The very high reactivity of this photoproduct towards biological substrates (*i.e.* proteins and lipids) could be the reason of the lack of its detection.