Aberrant expression of the epidermal tight junction protein Claudin-1 in polymorphic light eruption

671

The function of Langerhans cells is intrinsically regulated by urocanic acid

A Schwarz,[1] L Eckhart,[2] E Tschachler[2] and T Schwarz[1] *1 Dermatology, University of Kiel, Kiel, Germany and 2 Dermatology, University of Vienna, Vienna, Austria*

Cis-urocanic acid (cUCA), the UVB-induced isomer of trans-(t)UCA, is known to absorb UVB on the one hand but to mediate UVB-induced immunosuppression on the other hand. To assess which of these opposite activities is dominating in vivo we utilized histidinemic (HIS) mice which due to a lack of histidase have decreased levels of endogenous UCA in the skin. HIS and wild type (WT) mice were exposed to UVB on their shaved backs for 4 days. On day 5, the mice were sensitized with dinitrofluorobencene (DNFB) and ear challenge was performed 5 days later. UVB significantly suppressed sensitization in WT but only marginally in HIS mice. To investigate the reason for this difference, Langerhans cells (LC) were isolated from HIS and WT mice and labelled with DNBS, the water soluble analog of DNFB. Cells were injected subcutaneously (s.c.) into naive WT mice which were ear challenged 5 days later. In contrast to recipients of WT-LC, recipients of HIS-LC showed a pronounced ear swelling response to challenge, indicating that HIS-LC have a higher capacity of antigen presentation than WT-LC. FACS analysis revealed low expression of MHCII on WT-LC, but high levels on HIS-LC. To further study the effect of UCA on antigen presentation, bone marrow derived dendritic cells (BMDC) were incubated either with c- or tUCA, treated with DNBS and injected s.c. into naive mice which were challenged 5 days later. Injection of DNBS-treated BMDC induced sensitization against DNFB in the recipients. The sensitization was partially suppressed in recipients of cUCA-treated BMDC and, surprisingly, completely suppressed in recipients of tUCA-treated BMDC. Accordingly MHC-II and B7-2 expression were downregulated by cUCA and to an even larger degree by tUCA. Together, these results indicate that UCA may represent an endogenous mediator which controls the antigen presenting capacity of LC. This adds to the recent concept that LC contribute more to downregulation than induction of immune responses. UCA may be critically involved in this process.

672

The UVA component of solar simulated radiation differentially degrades dermal proteins associated with photoageing

SA Thurstan,[1] NK Gibbs,[1] C Baldock,[2] AS Weiss,[3] CE Griffiths,[1] RE Watson[1] and MJ Sherratt[1] *1 Institute of Inflammation and Repair, The University of Manchester, Manchester, United Kingdom, 2 School of Molecular Bioscience, The University of Sydney, Sydney, NSW, Australia and 3 Faculty of Life Sciences, The University of Manchester, Manchester, United Kingdom*

Chronic exposure to ultraviolet (UV) radiation causes remodelling of the dermal extracellular matrix (ECM) leading to photoageing. This remodelling may be driven by cell-derived proteases and/or photochemical mechanisms. We have previously shown that the amino acid composition of dermal ECM proteins correlates with their relative susceptibility to degradation by broadband UVB radiation. UV-chromophore-rich proteins such as fibrillin and fibronectin were preferentially degraded but not chromophore-poor collagen I and tropoelastin. In this study, we determine whether physiological doses of the UVA (315-400nm) component of solar radiation are capable of differentially degrading these ECM proteins. Suspensions of bovine collagen I and fibronectin, recombinant tropoelastin and human dermal fibroblast-derived fibrillin microfibrils were exposed to full spectrum Solar Simulated Radiation (SSR; 290-400nm: 0.8, 7.7 and 15.4J/cm2 and UVA (315-400nm): 1, 10 and 20J/cm2. The effects of SSR and UVA exposure on molecular structure were determined by reducing SDS-PAGE (collagen I, tropoelastin and fibronectin) and atomic force microscopy (fibrillin). Neither SSR nor UVA radiation affected the electrophoretic mobility of chromophore-poor collagen I and tropoelastin. However, both UV spectra induced dose-dependent aggregation of fibronectin (SSR: r2 0.99, UVA: r2 0.98). Similarly, both SSR and UVA radiation also significantly reduced the bead-to-bead distance of fibrillin microfibrils (SSR: mean±SD, 56.6±9.7nm [0J/cm2], 53.2±10.3nm [15.4J/cm2], $p<0.001$; UVA: 58.2±9.6nm [0J/cm2], 56.0±15.9nm [20J/cm2], $p<0.001$). These data suggest that, in contrast to structural proteins (collagen I and elastin), adhesive glycoproteins (fibronectin and fibrillin) may be susceptible to direct damage by physiological doses of the UVA component of solar radiation in vivo.

673

Galectin-7, induced by *cis*-urocanic acid and ultraviolet B irradiation, down-modulates cytokine production by T lymphocytes

M Nakamura and R Yoshiki *Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu, Japan*

Urocanic acid (UCA) is an epidermal chromophore that undergoes *trans* to *cis* isomerization after UVB irradiation. *cis*-UCA is a potent inhibitor of cutaneous acquired immunity. However, its underlying molecular mechanism still remains unclear. Here, we explored genes which are up-regulated by *cis*-UCA in normal human epidermal keratinocytes (NHEK) by DNA microarray analysis and investigated their roles *in vitro* and *in vivo* using T lymphocyte cell line, Jurkat cells, and C3H/HeJ mice, a mouse model of T-cell mediated autoimmune alopecia, alopecia areata. DNA microarray experiments revealed that *cis*-UCA increased the expression of a gene encoding a β-galactoside-binding lectin, galectin-7, *LGALS7B*. Quantitative real-time RT-PCR analysis showed that the expression of galectin-7 was enhanced by *cis*-UCA and UVB irradiation, but not by *trans*-UCA. Immunohistochemical study demonstrated that galectin-7 was highly expressed in the epidermis in the patients with actinic keratosis. To explore a roles of galectin-7 in the control of cytokine production by T lymphocytes, we cultured Jurkat cells with recombinant galetin-7. Galectin-7 administration inhibited the expression of interleukin-2 (*IL2*) and interferon-γ (*IFNG*) mRNA by Jurkat cells. Enzyme linked immunosorbent assay(ELISA) revealed that the IL-2 concentration of culture sup of Jurkat cells was down-regulated by galectin-7. Transwell-assay demonstrated the down-modulation of the movement of Jurkat cells by the addition of galectin-7. To elucidate the effects of galectin-7 *in vivo*, we injected recombinant galectin-7 into alopecic areas of C3H/HeJ mice, a mouse model of alopecia areata. The local injections of galectin-7 ameliorated alopecia in C3H/HeJ mice. Taken together, these data suggested that galectin-7 may play important roles in down-regulating the functions of T lymphocytes after UVB irradiation and can be developed into novel immunosuppressive therapies for inflammatory skin diseases.

674

MicroRNA-34a exhibits different responses to ultraviolet radiation and ionising radiation

E Vavatsikou,[1] C Pickard,[1] T Sanchez-Elsner[2] and E Healy[1] *1 Dermatopharmacology, University of Southampton, Southampton, United Kingdom and 2 MicroRNA Laboratory, University of Southampton, Southampton, United Kingdom*

Exposure to ultraviolet radiation (UVR) causes DNA damage in skin, and leads to post-transcriptional elevation of p53 protein. p53 is also upregulated by other DNA damaging agents, such as ionising radiation, and results in transactivation of a variety of downstream signalling pathways, including microRNA-34a (miR-34a). miRs are small non-coding RNA transcripts that act as gene silencers in a post-transcriptional manner, and the increase in miR-34a after ionising radiation has been reported to cause a reduction of sirtuin 1 (SIRT1), leading to apoptosis. In the current study, we examined for alterations in miR-34a following exposure to UVB. Human primary keratinocytes (HPK), HaCaT keratinocytes (containing mutant p53), HCT116 (human colonic carcinoma cell line with wild type p53), and ex vivo human skin were exposed to doses of UVB (151.68mJ/cm2 TL12 lamp), and separately to ionising radiation (20Gy), which caused significant upregulation of p53 protein in each cell type / tissue sample (Wilcoxon matched-pair signed rank test; $p<0.05$). As expected, UVB and ionising radiation increased the p53 downstream effector molecule, p21WAF1, significantly in HPK, HCT116 and human skin ($p<0.05$), but not in HaCaT cells. Whereas miR-34a became elevated in HPK, HCT116 and human skin after ionising radiation ($p<0.05$), miR-34a did not increase in any of these samples after UVB, but instead significantly decreased in HPK and HCT116 cells ($p=0.05$ and 0.01 respectively) and remained stable in skin following UVB exposure. Furthermore, immunoblotting demonstrated that ionising radiation caused a significant reduction in SIRT1 protein ($p<0.05$), whereas SIRT1 did not decrease after UVB irradiation. Thus, despite similar elevation of p53 following exposure to ionising radiation and UVR, the p53-downstream effector miR-34a responds differently to these two stimuli, with ionising radiation causing elevation of miR-34a but UVB failing to upregulate miR-34a.

675

Tropisetron via α7 nicotinic acetylcholine receptors modulates the inflammatory response of human epidermal keratinocytes after exposure to UVB light or TNF-α

A Stegemann and M Böhm *University of Münster, Münster, Germany*

Ultraviolet (UV) light has a key role in skin carcinogenesis. Proinflammatory cytokines such as tumor necrosis factor (TNF)-α mediate some of the inflammatory responses of epidermal cells after UVB treatment and sustained activation of their signaling pathways are implicated in photocarcinogenesis. There is increasing evidence for a modulatory role of serotonin (5-hydroxytryptamine, 5-HT)-mediated pathways in the control of inflammatory responses in various organs of the human body. Using tropisetron, a 5-HT-receptor (5-HT-R) modulating agent approved as an antiemetic, we investigated its effect on UVB- and TNF-α-mediated induction of proinflammatory mediators such as interleukin (IL)-6, IL-8 and cyclooxygenase-2 (COX-2) in human epidermal keratinocytes (NHK). Tropisetron attenuated UVB- and TNF-α-induced IL-6, IL-8 and COX-2 mRNA expression as shown by real-time RT-PCR. This suppressive effect of tropisetron on UVB- and TNF-α-mediated increase of IL-6 and IL-8 expression was confirmed at protein level in NHK. Mechanistically, tropisetron reduced TNF-α-mediated nuclear translocation of p65/NF-κB but neither affected p38-signaling nor IκBα-degradation. Since tropisetron was previously shown to also act on α7 nicotinic acetylcholine receptors (α7nAchRs) we performed expression analyses of the putative tropisetron receptors in NHK. In support of a 5-HT-receptor-independent action of the drug, the 5-HT3-R and 5-HT4-R were undetectable in NHK at RNA and protein level. However, the expression of the closely related α7nAchR was detected in NHK suggesting that this receptor could be the mediator of the anti-inflammatory effect of tropisetron in NHK. Moreover, an α7nAChR antagonist neutralized whereas a specific α7nAchR agonist mimicked the effect of tropisetron on UVB-induced signals in NHK. In summary, our results highlight an anti-inflammatory potential of tropisetron in epidermal keratinocytes and point towards future strategies in the treatment of inflammatory skin diseases via targeting the α7nAChR.

676

PPARγ is involved in the beneficial effect of azelaic acid against photo-induced skin senescence

S Briganti, E Flori, A Mastrofrancesco, G Cardinali, D Kovacs, M Ludovici, E Camera and M Picardo *Cutaneous Physiopathology Laboratory and Integrated Center of Metabolomics, IRCSS San Gallicano Dermatology Institute, Rome, Italy*

Azelaic acid (AzA) has been used for the treatment of inflammatory skin diseases, such as acne and rosacea. An improvement of skin texture has been observed after long time treatment with AzA. We previously unrevealed that anti-inflammatory activity of AzA involves a specific activation of PPARγ, a nuclear receptor that plays a relevant role in inflammation and even in ageing processes. Since rosacea has been considered as a photo-aggravated disease, we investigated the ability of AzA to counteract stress-induced premature senescence (SIPS). We employed a SIPS model based on single exposure of human dermal fibroblasts (HDFs) to UVA and 8-methoxypsoralen (PUVA), previously reported to activate a senescence-like phenotype, including long-term growth arrest, flattened morphology, and increased synthesis of matrix metalloproteinases (MMPs) and senescence-associated- β-galactosidase (SA-β-gal). We found that PUVA-treated HDFs grown in the presence of AzA rescued the elongated cell shape, and reduced of MMP-1 release and SA-β-galactosidase-positive cells. Moreover, AzA determined a reduction of ROS generation, an up-modulation of antioxidant enzymes and a decrease of cell membrane lipid damages in PUVA-treated HDFs. Further evidence of AzA anti-senescence effect were repression of p53 and p21, increase of type I pro-collagen, and abrogation of the increased expression of growth factors, such as HGF or SCF. Interestingly, PUVA-SIPS showed a decreased activation of PPARγ and AzA counteracted this effect, suggesting that AzA effect involves PPARγ modulation. All together these data showed that AzA interferes with PUVA-induced senescence-like phenotype and its ability to activate PPAR-γ provides relevant insights into the anti-senescence mechanism.

677

CPD-Dependent Gene Expression Changes In Photolyase MRNA-Transfected Human Keratinocytes Irradiated By UVB

G Boros,[1] E Miko,[1] E Emri,[1] G van der Horst,[2] H Muramatsu,[3] D Weissman,[4] I Horkay,[1] G Emri,[1] K Kariko[3] and ÉVA Remenyik[1] *1 Department of Dermatology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary, 2 Department of Genetics, Center for Biomedical Genetics, Erasmus University Medical Center, Rotterdam, Netherlands, 3 Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA and 4 Department of Medicine, University of Pennsylvania, Philadelphia, PA*

Exposure of skin cells to solar UV induces substantial change in the pattern of actively transcribed genes including those involved in DNA repair, cell growth, apoptosis and immune responses. The mechanism of transcriptional regulation triggered by UVB is not fully elucidated. One major type of the UVB-induced DNA damages is cyclobutane pyrimidine dimer (CPD), which mediates several cellular effects of UVB. Recently, we have established a model system in which 90% of UVB-induced CPD could be removed from keratinocytes by photolyase expressed ectopically from transfected mRNA. Human keratinocytes were exposed to 20 mJ/cm2 UVB at 12 h posttransfection when the translation level of in vitro-transcribed photolyase mRNA reached the maximum. Using oligonucleotide microarrays, global gene expression profiles were determined. At 6 h following UVB irradiation, 696 genes were significantly upregulated and 1372 were downregulated. Interestingly, more than half of these genes were CPD-dependent, associated primarily with gene expression, tissue morphology and DNA repair. Induction of transcription regulators, including FOXO and SMAD that could drive the cellular responses to CPDs was also measured. In conclusion, removal of CPDs in primary keratinocytes by ectopic expression of photolyase from transfected mRNA is a novel approach to discern the CPD-dependent and -independent effects of UVB and these data will lead to a better understanding of the mechanism of cutaneous defense against sun exposure.

678

Involvement of MIF in Basement Membrane Damage in Chronically UVB-exposed Skin in Mice

Y Yoshihisa,[1] O Norisugi,[1] K Matsunaga,[1] J Nishihira[2] and T Shimizu[1] *1 Dermatology, University of Toyama, Toyama, Japan and 2 Medical Information, Hokkaido Information University, Ebetsu, Japan*

Solar ultraviolet (UV) exposure is known to induce premature aging of the skin, which is mediated by the matrix metalloproteinase (MMPs) activity. This photoaged skin is biochemically characterized by a predominance of abnormal elastic fibers and a dramatic decrease in dermal collagen. The basement membrane at the dermal-epidermal junction is also damaged during the wrinkle formation process. Macrophage migration inhibitory factor (MIF) is a pluripotent cytokine that plays an essential role in the pathophysiology of allergic inflammation and UV irradiation. In this study, we report the relationship between the expression of MIF, UVB-induced MMPs activities and skin changes in mice. The backs of MIF transgenic (Tg) and wild-type (WT) mice were exposed to UVB three times a week for 10 weeks. We found that the back skin of the MIF Tg mice had higher MIF, MMP-2 and MMP-9 expression levels than observed in WT mice, and this occurred in a time-/exposure-dependent manner. Moreover, we observed a decrease in the expression of type IV collagen and increased basement membrane damage in the exposed skin of the MIF Tg mice after 10 weeks of UVB exposure. We therefore concluded that the MIF-mediated enhancement of collagen degradation and basement membrane damage occurs mainly through the activation of MMPs expression in mouse skin following exposure to UVB radiation. We next examined the effect of MIF on UVB-induced MMPs in keratinocytes and fibroblast cells of both MIF Tg and WT mice. MIF induced an increase in the MMPs expression, including MMP-9 in the keratinocytes and MMP-2 in the fibroblasts. In conclusion, MIF is considered to be an important factor involved in the UVB-induced MMPs activation and basement membrane damage in mouse skin.

679

Suppression of UV-induced Wrinkle Formation by Induction of HSP70 Expression in Mice

T Mizushima and M Matsuda *Faculty of Pharmacy, Keio University, Tokyo, Japan*

UV-induced wrinkle formation due to the degeneration of the ECM is major dermatological problem, in which abnormal activation of MMPs and elastases play important roles. HSP70 has cytoprotective and anti-inflammatory activities. In the present study, we examined the effect of HSP70 expression on UV-induced wrinkle formation. Heat treatment of the dorsal skin of hairless mice induced the expression of HSP70. The long-term repeated exposure to UVB induced epidermal hyperplasia, decreased skin elasticity, degeneration of ECM and wrinkle formation, which could be suppressed in mice concomitantly subjected to heat treatment. The UV-induced epidermal hyperplasia, decreased skin elasticity and degeneration of ECM were less apparent in transgenic mice expressing HSP70 than in wild-type mice. UV-induced fibroblast cell death, infiltration of inflammatory cells and activation of MMPs and elastase in the skin were also suppressed in the transgenic mice. This study provides the first evidence for an inhibitory effect of HSP70 on UV-induced wrinkle formation. The results suggest that this effect is mediated by various properties of HSP70, including its cytoprotective and anti-inflammatory activities. We propose that HSP70 inducers used in a clinical context could prove beneficial for the prevention of UV-induced wrinkle formation.

680

Aberrant expression of the epidermal tight junction protein Claudin-1 in polymorphic light eruption

EJ Pond, CA O'Neill, LE Rhodes and NK Gibbs *Dermatological Sciences, University of Manchester, Manchester Academic Health Sciences Centre, Manchester, United Kingdom*

The exact aetiology of polymorphic light eruption (PLE) is unknown but is thought to involve a delayed-type immunological reaction to an unknown endogenous or exogenous 'photoallergen' formed after skin exposure to ultraviolet radiation (UVR). A recent study has reported compromised skin barrier function in PLE. Claudin-1 (CLD-1) is critical to tight junction formation and skin barrier function, down-regulates keratinocyte proliferation and has reduced epidermal expression after acute UVR exposure. We therefore investigated CLD-1 protein expression and epidermal thickness in non-irradiated (NI) and UVB irradiated skin of patients with PLE (n=6) and healthy controls (n=6). Photo-protected buttock skin was exposed to 200 mJ/cm^2 UVB from a broadband Philips TL-12 source. 24h later, biopsies were taken from NI and UVB irradiated skin and CLD-1 expression levels measured in cryosections using quantitative immunofluorescence. In NI skin, CLD-1 expression was significantly reduced in PLE patients ($p<0.001$) compared to controls. This was particularly evident in the *stratum spinosum*. In healthy skin there was a highly significant reduction ($p<0.001$) in CLD-1 expression 24h after UVB exposure, which was most prominent in basal layer keratinocytes. In contrast, there was no loss of CLD-1 expression in PLE after UVB exposure. The mean (+/- SEM) thickness of NI skin was higher In PLE subjects, (72.43 ±1.76μm) than in controls (68.98 ± 1.23μm) but this did not reach statistical significance. Following UVB exposure there was a 19% increase of epidermal thickness in controls (82.14 ±1.74μm; $p<0.001$) compared to only a 9% rise in PLE patients (79.66 ±1.77μm; $p<0.05$). These findings suggest that reduced CLD-1 expression and aberrant UVB-induced skin thickening responses may be aetiological features of PLE.

681

Biological effects of longwave UVA (UVA-1) on human reconstructed skin model

C Marionnet, F Lejeune, C Pierrard and F Bernerd *L Oreal Research & Innovation, Clichy, France*

Frequent sun exposure leads to skin alterations resulting in photoaging or photocarcinogenesis, involving both UVB (280-320 nm) and short and longwave UVA (UVA-2, 320-340 nm and UVA-1, 340-400 nm). UVA-1 which represents the majority of UV wavelengths reaching earth level generate reactive oxygen species and have been shown to induce deleterious effects including immunosuppression and carcinogenesis, as shown by animal and human in vivo studies. In the present study, the biological impact of UVA-1 was assessed through morphological, biochemical and gene expression analysis using a 3D reconstructed skin model including both a fully differentiated epidermis and a dermal equivalent containing human fibroblasts. Forty-eight hours after exposure to 40 J/cm2 UVA-1, morphological changes were detected, notably the disappearance of superficial dermal fibroblasts and a mild alteration of epidermal differentiation. These alterations may be due to oxidative stress revealed immediately following exposure using the DCFH-DA probe. In parallel, an increase in the level of several secreted proteins (cytokines, chemokines, MMPs) could be identified using ELISA detection. Gene expression was analyzed using Affymetrix microarrays 6 hours after reconstructed skin exposure to 40J/cm2 UVA-1. Fibroblasts and keratinocytes showed altered expression (1.5 fold, adjusted p value <0.001) of 461 and 480 mRNA levels, respectively, indicating a molecular impact of such wavelengths from the surface to the depth of the skin model. Q-PCR experiments confirmed, at mRNA level, alterations of genes related to oxidative stress, inflammation, epidermal differentiation or extracellular matrix. Our data shows that UVA-1 radiation has a biological impact on reconstructed skin model affecting gene and protein expression in both fibroblasts and keratinocytes. These results give new insights into the biological events specifically induced by UVA-1 exposure and highly support the need for an adequate longwave UVA photoprotection.

682

Effect of photodynamic therapy (PDT) on regulatory T cells in patients with actinic keratoses vs. esophageal squamous cell carcinoma

E Reginato,[1] J Lindenmann,[2] A Gruber-Wackernagel,[1] G Mayer,[1] I Bambach,[1] N Schweintzger,[1] C Langner,[3] F Smolle-Jüttner[2] and P Wolf[1] *1 Department of Dermatology, Medical University of Graz, Graz, Austria, 2 Department of Thoracic Surgery, Medical University of Graz, Graz, Austria and 3 Institute of Pathology, Medical University of Graz, Graz, Austria*

Photodynamic therapy (PDT) has become an established clinical modality for the treatment of various types of cancer. PDT consists of the administration of a photosensitizer (PS) to the patient, followed by irradiation of the diseased area with a light of appropriate wavelength. When the PS absorbs the light, it converts molecular oxygen to highly reactive single oxygen (1O2) that causes apoptosis and necrosis of illuminated tumor cells, shuts down tumor microvessels, and induces an acute inflammatory response. Moreover, these events could lead to the stimulation of a systemic adaptive anti-tumor immune response. Previous work in experimental models has revealed that depleting regulatory T cells (Tregs) can potentiate the efficacy of PDT. We therefore became interested to investigate the immunological changes induced by PDT and its effect of PDT on levels and function of Tregs (CD4+CD25highCD127lowFoxP3+ cells). Such an effect may be of importance in PDT-treated patients with esophageal squamous cell carcinoma (ESCC), in whom prolonged survival has been reported after multi-treatment approaches. To investigate the hypothesis blood is collected from patients with invasive ESCC or patients with (non-invasive) actinic keratoses (AK) as control before PDT, 7 and 14 days after treatment. Treg levels are quantified by FACS and Treg function by co-culture proliferation assays with T effector cells (CD4+CD25lowCD127highcells). Our results so far indicate that PDT may inhibit the suppressive capacity of systemic Tregs from ESCC but not from AK patients. Treg levels, however, appear to be are highly variable among patients. A better understanding of the immunological events linked to PDT are of great importance for optimization of the approach and development of strategies to improve the ultimate outcome in treated patients.

683

Differential effects of excimer light (308nm) and narrow-band UVB (311nm) on pigment cell development: Novel insights for better phototherapeutic strategy

H Yu and CE Lan *Dermatology, Kaohsiung Medical University, Kaohsiung, Taiwan*

Phototherapy is an important treatment option for vitiligo. Narrow-band UVB (NBUVB: 311nm) has been considered as the treatment of choice. Recently, excimer light (308nm) has been introduced as another option for treating vitiligo. Emitting lights at similar wavelength but at much higher irradiance, excimer light has been shown to induce repigmentation of vitiligo more rapidly and efficiently as compared to NBUVB. Moreover, the lesions that failed NBUVB therapy also showed response to excimer treatment. Although the wavelength of excimer and NBUVB light differed by only few nanometers, the mechanisms responsible for the different clinical effects observed remained obscure. In our in vitro studies, we demonstrated that at similar doses, excimer light imparted different biological effects on primitive pigment cells as compared to its NBUVB counterpart. Both nuclear DNA and cytoplasmic tryptophan are recognized photoacceptors for UVB treatment. After irradiation at comparable fluences, excimer light was able to induce primitive pigment cell differentiation through efficient activation of cytoplasmic pathway that involved formation of tryptophan derivate to activate aryl hydrocarbon receptor-related cascade while NBUVB failed to do so. Reduction of excimer light irradiance by external filter abrogated the effects of excimer light on primitive pigment cells at equivalent doses. In addition, gene silencing of aryl hydrocarbon receptor also reduced the effect of excimer light on primitive pigment cell development. In summary, excimer and NBUVB lights are both effective for inducing vitiligo repigmentation but via different pathways. The difference in irradiance appeared to play an important role accounting for the more rapid clinical response observed for excimer light treated vitiligo lesions.

684

UV dose-response and dose-delivery in hairless pigmented mice

CM Lerche,[1] K Togsverd-Bo,[1] PA Philipsen,[1] T Poulsen[2] and H Wulf[1] *1 Department of Dermatology, Bispebjerg University Hospital, Copenhagen, Denmark and 2 Department of Pathology, Hospital of Southern Jutland, Soenderborg, Denmark*

In humans it is not clear if few very high ultraviolet radiation (UVR) exposures are more carcinogenic than multiple smaller exposures. Previous studies suggest that time-dose reciprocity is absent because at equal cumulative doses intermittent high dose exposure is less carcinogenic. However, these studies are conducted in hairless albino mice in contrast to our mouse model, which can develop pigmentation after UVR. Humans do also develop pigmentation after UVR so we find it very relevant to conduct this type of study in a pigmenting mouse model. We made a dose-response study from 0.6-4 Standard Erythema Doses (SED) and a dose-delivery study investigating whether a few high exposures of UVR are more photocarcinogenic than multiple smaller exposures. Female hairless C3.Cg/TifBomTac immunocompetent mice (n=351) were included in the study. Treatment schedules were 0, 0.6, 1.2, 2, 3, or 4 SED 3/week (Part I) and 2 SED 6/week, 3 SED 4/week, 4 SED 3/week, or 6 SED 2/week (Part II). All mice were irradiated until development of 3 tumours of 4 mm. Part I showed a linear dose-response curve between log time and log dose. Part II showed that the median time to the first tumour did not differ between the 4 groups. The median time to the second tumour was significantly different between 2 SED 6/week and 6 SED 2/week (169 vs 190 days, p=0.014), and the median time to the third tumour was significantly different between 4 SED 3/week and 6 SED 2/week 4 (183 vs 197 days, p=0.005), but not between the rest of the groups. In conclusion, this study confirms the expected relationship between dose and carcinogenic response. The dose-delivery results showed no difference in time to the first tumor. However, if we include the results for development of the second and third tumour there is a tendency to a more carcinogenic effect at fractionated delivery. This could be due to the fact that mice receiving few high exposures develop pigmentation faster than mice receiving smaller multiple exposures.

685

Narrow-band ultraviolet B course improves vitamin D balance in dialysis patients on oral vitamin D supplementation

MJ Ala-Houhala,[1] K Vähävihu,[2] H Kautiainen,[3] T Hasan,[2] E Snellman,[4] Y Dombrowski,[5] J Schauber,[5] H Saha[6] and T Reunala[1] *1 Medical School, University of Tampere, Tampere, Finland, 2 Department of Dermatology, Tampere University Hospital, Tampere, Finland, 3 Unit of Primary Health Care, Kuopio University Hospital, Kuopio, Finland, 4 Department of Dermatology, Päijät-Häme Central Hospital, Lahti, Finland, 5 Department of Dermatology and Allergy, Ludwig-Maximilians-University, Munich, Germany and 6 Department of Internal Medicine, Tampere University Hospital, Tampere, Finland*

Narrow-band UVB (NB-UVB) treatment heals psoriasis and increases serum 25-hydroxyvitamin D (25(OH)D) concentration. We have shown that a short NB-UVB course increases 25(OH)D more than daily oral vitamin D supplementation. Chronic kidney disease (CKD) patients are prone to vitamin D insufficiency despite oral vitamin D supplementation. We studied whether a NB-UVB course could improve their vitamin D balance. 14 CKD patients (mean age 53.6 years) on haemodialysis and 15 healthy subjects (46.1 years) received oral cholecalciferol 20 μg daily before, during and after a NB-UVB course given thrice a week with a Waldmann UV cabin. The cumulative UVB dose was 4.45 J/cm2 (26.2 SED). Serum 25(OH)D was measured by radioimmunoassay. mRNA expression of CYP27B1, i.e. 1-α-hydroxylase which is needed for hydroxylation of vitamin D into its active metabolite, was examined from skin biopsy specimen. At baseline the mean 25(OH)D was 57.7±18.2 nmol/L in the CKD patients and 74.3±14.8 nmol/L in the healthy subjects. The increase of 25(OH)D after 9 NB-UVB exposures was 14.0 nmol/L (95% CI 8.7 and 19.3; $p<0.001$) and 17.0 nmol/L (13.6 and 20.3; $p<0.001$), respectively. One month after the NB-UVB course 25(OH)D levels were still significantly higher than at baseline. CKD patients showed markedly increased CYP27B1 mRNA expression at baseline and after NB-UVB course compared to healthy subjects. This study shows that a short NB-UVB course is an easy way to improve vitamin D balance in CKD patients who already have oral vitamin D supplementation. The increased CYP27B1 levels in the CKD patients suggest that the loss of renal activity of this enzyme is at least partly compensated in the skin.

686

Visible blue light effects on denditric cells

S Lembo,[1] A Balato,[1] M Cantelli,[1] M Schiattarella,[1] E Ciaglia[2] and G Monfrecola[1] *1 Dermatology, University Federico II, Naples, Italy and 2 Pharmaceutical and Biomedical Sciences, University of Salerno, Fisciano (Sa), Italy*

Visible blue light has a wavelength spectrum from 400 nm to 475 nm with a peak at 420 nm. Theoretically, blue light could induce biological effects comparable to UVA radiation, since wavelengths of blue light are closely related to the UVA spectrum, hence effects on the skin have been reported. In the present study, the effects of blue light on dendritic cells (DCs) maturation and pro-inflammatory cytokines production are investigated. Monocyte derived dendritic cells were isolated from peripheral blood mononuclear cells, using anti-CD14 conjugate magnetic microbeads. To assess whether blue light was able to modify DCs differentiation and maturation process, CD 14+ cells were irradiated with 2.5; 5; 10 and 15 J/cm° of blue light. Thereafter immature DCs (iDCs) were generated through incubation with granulocytes monocytes-colony stimulating factor (GM-CSF) and interleukin (IL)-4. Finally mature DCs were obtained after incubation of iDCs with LPS and were identified through flow cytometry using anti CD83 and CD86 antibodies. The production of pro-inflammatory cytokines, such as IL-6 and TNF-α, was also assessed by intracitoplasmatic immunofluorescence in irradiated and unirradiated cells. The exposure to blue light did not alter the ability to terminal differentiate in mature DCs. Blue light treatment reduced, in a dose dependent manner, the production of IL-6 and TNF-α promoted by LPS in the mature DCs. This report indicates that blue light has anti-inflammatory effects on DCs, without altering their differentiation process.

687

Soybean extract showed modulation of retinoic acid-related genes expression of skin and photo-protective effects in keratinocytes

Y Na, N Park, J Park, Y Kang, J Bae, H Lee, M Yeom and J Cho *Skin Research Institute, Amorepacific R&D center, Yongin, Republic of Korea*

Soy extracts are well known medicinal and nutritional ingredient, and showed benefits on human skin including depigmenting or anti-aging effects. Although wrinkle decreasing effects of retinoids on skin as an anti-aging ingredient, retinoids application causes photo-sensitive response such as skin irritation. Thus their daytime usage was not recommended. The aim of this study is the investigation of soybean extract activities as an anti-aging ingredient compared to retinoids. Soybean extract decreased relative ratio of MMP-1/TIMP-1 mRNA level as much as retinoic acid in normal human fibroblasts. It also affected mRNA level of HAS2 and CRABP2 in normal human keratinocytes. Furthermore, we investigated its effect on mRNA expression of histidase, an enzyme that converts histidine into urocanic acid, the main UV light abosorption factor of the stratum corneum. Unlike dramatic decrease of histidase mRNA expression by retinoic acid, the effect of soybean extract on histidase gene expression was less than retinoic acid in normal human keratinocytes. Also soybean extract treatment inhibited UVB-induced cyclobutane-pyrimidine dimer formation dose-dependently in normal human keratinocytes. In this study, we found that soybean extract modulated retinoic acid-related genes and showed photo-protective effects. Our findings suggest that soybean extract might be an anti-aging ingredient that can be safely used under the sunlight.

688

Global gene expression profiles in lymphocyte subsets during extracorporeal photochemotherapy

U Just,[1] G Klosner,[1] F Klinglmueller,[2] M Bilban,[3] Z Kuzmina,[4] H Greinix,[4] R Knobler[1] and F Trautinger[5] *1 Dept of Dermatology, University of Vienna, Vienna, Austria, 2 Center for Medical Statistics, University of Vienna, Vienna, Austria, 3 Dept of Laboratory Med, University of Vienna, Vienna, Austria, 4 Dept of Internal Med, University of Vienna, Vienna, Austria and 5 Karl Landsteiner Institute for Dermatol Research, St Pölten, Austria*

Extracorporeal photochemotherapy (ECP) is a treatment for cutaneous T cell lymphomas, graft-versus-host disease (GvHD), organ rejection, systemic sclerosis, and other immune-mediated disorders. During ECP peripheral blood leukocytes are exposed to 8-MOP and UVA prior to reinfusion to the patient. Only limited information is available on mechanisms of action of ECP and its influence on gene expression in treated cells is currently unknown. Affymetrix® Human Genome U133 Plus 2.0 Arrays were used to compare global gene expression profiles in CD4+ and CD8+ lymphocytes before and after ECP. 6 female patients with chronic GvHD were included in the study. CD4+ and CD8+ lymphocyte subsets were isolated before and immediately after exposure to 8-MOP/UVA during a standard ECP treatment cycle. Total RNA was isolated from each cell sample, processed and analyzed according to standard procedures. Exposure to 8-MOP/UVA during ECP induces an immediate and specific molecular response in CD4+ and CD8+ lymphocytes in patients with GvHD. There was a striking difference between CD4+ and CD8+ subsets in numbers of regulated genes. In CD4+ cells 101 genes showed significant regulation (48 up, 53 down; false recovery rate adjusted $p<0.01$). Applying the same criteria in CD8+ cells only 6 down regulated genes could be detected. The results of these experiments provide for the first time a database of ECP regulated genes which is not based on a preformed hypothesis. Thus it provides an unbiased look on molecular events that might underlie the therapeutic efficacy of ECP. Confirmation and further functional analysis of genes detected in this study as well as extension of these experiments to other leukocyte subsets and other diseases treated with ECP will help to identify possible predictors for response to ECP.

689

Flat spectrum sun screen protects against ultraviolet radiation induced damage to key extracellular matrix components in vitro

P Costello,[1] SA Thurstan,[1] CE Griffiths,[1] M Bell,[2] M Brown,[2] AK Langton,[1] MJ Sherratt[1] and RE Watson[1] *1 Institute of Inflammation and Repair, Manchester Academic Health Science Centre, The University of Manchester, Manchester, United Kingdom and 2 Alliance Boots, Nottingham, United Kingdom*

Ultraviolet radiation has profound effects on the structure and function of extracellular matrix components. We have previously demonstrated that molecules rich in amino acid UV-chromophores (fibrillin and fibronectin), are susceptible to physiological doses of solar simulated radiation (SSR). In the current study we have investigated whether a flat-spectrum sunscreen (similar absorbance at all wavelengths) is capable of protecting fibrillin and fibronectin from degradation by SSR. Suspensions of fibronectin and fibrillin microfibrils derived from photoprotected human dermis were exposed to a single dose of SSR (290-400nm; 23.7J/cm2): i) in the absence of any protection (SSR); ii) covered by a quartz plate coated with vehicle (1mg/cm2; SSR+V); iii) covered by a quartz plate coated with a flat-spectrum sunscreen (1mg/cm2; SSR+SS) (n=3). The effect of SSR on molecular structure was determined by atomic force microscopy (fibrillin) and native PAGE (fibronectin). Following irradiation, fibrillin microfibril structure underwent profound remodelling in SSR and SSR+V samples which was partially prevented by the flat-spectrum sunscreen. Microfibril periodicity in the unexposed sample was unimodally distributed (centred at 55.4nm). However, irradiation (in the absence of sunscreen) abolished this single population (bimodal with peaks centred at 45.3 and 66.1nm). In SSR+SS samples, this molecular remodelling was partially prevented (bimodal with peaks at 51.9 and 65nm). SSR exposure induced significant aggregation of fibronectin ($p=0.005$), which was abrogated by the flat-spectrum sunscreen ($p=0.033$) but not the vehicle control ($p=0.754$). We conclude that a flat spectrum sunscreen can reduce direct UV-mediated damage of key dermal molecules and thereby in part prevent UV-induced skin damage.

690

Investigating the Protective Potential of the Antioxidant Tiron in Human Skin Cells Exposed to Ultraviolet Radiation

EV Russell, JA Latimer, A Oyewole and MA Birch-Machin *Dermatological Sciences, Newcastle University, Newcastle-upon-Tyne, United Kingdom*

Exposure to ultraviolet radiation (UVR), the major component of sunlight, can lead to skin cancer and premature ageing. The UVB component of UVR can induce damage directly through DNA absorption and generation of cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs). UVA, the longer wavelength component of UVR, can also induce CPD and 6-4PP formation through an alternative indirect route of reactive oxygen species (ROS) production. Our previous work has demonstrated the ROS scavenging ability of synthetic vitamin E antioxidant Tiron. This current study aims to further investigate the protective potential of Tiron in terms of photoproduct induction as measured by immunofluorescence. Human neonatal dermal fibroblast (HDFn) cells were irradiated with Cleo Performance (~120 J/cm^2, glass filtered UVA), TL12 (~3 J/cm^2, broadband UVB) or Arimed B (~72 J/cm^2, UVA/UVB) lamps, both with and without Tiron pre-incubation (optimum dose, removed prior to UVR exposure). 6-4PPs and CPDs were detected by immunofluorescence using the monoclonal antibodies 64M-2 and TDM-2 respectively. Results demonstrate that UVA causes a 1.5-fold increase (n=3, $P<0.0001$) in 6-4PPs and 3-fold increase (n=3, $P<0.0001$) in CPDs. On application of Tiron, levels of UVA-induced 6-4PPs and CPDs were reduced by ~20% (n=3, $P<0.0001$) and ~45% (n=3, $P<0.0001$) respectively, suggesting a moderate level of protection. Irradiation with UVB caused a 3-fold increase (n=3, $P<0.0001$) and 6-fold increase (n=3, $P<0.0001$) in 6-4PPs and CPDs respectively. Tiron demonstrated little protection against UVB with a ~2% decrease (n=3) in 6-4PPs and ~10% decrease (n=3) in CPDs. These observations have not been previously reported, and support previous findings that Tiron acts by a defensive mechanism of ROS scavenging as demonstrated by protection against UVA-induced damage only. This represents a platform to investigate other antioxidants of similar structure as protective agents against UVA-induced sun damage in humans.

691

Modulating effect of zinc on cutaneous UVB-response

E Emri,[1] E Miko,[1] G Nagy,[2] G Boros,[1] D Rozsa,[1] G Mocsai,[2] G Emri[1] and E Remenyik[1] *1 Department of Dermatology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary and 2 Department of Dermatology and Dermatological Allergology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary*

In the skin zinc deficiency induces necrolysis and cutaneous inflammation, furthermore is associated with impaired adaptive immunity. Metallothionein, the zinc buffering protein, has prognostic relevance in skin cancers and likely it has an immune-modulating effect like we found in our previous work in melanoma. It is well known that UVB has crucial role in the development and progression of skin cancer, however modulating effect of the intracellular zinc level on the gene expression related to UVB-response (DNA-repair, cell cycle regulation, apoptosis, and inflammation) is not known. We studied the expression pattern of several genes after ZnCl2 treatment (100 μM) and UVB (20 mJ/cm2) exposure in human keratinocyte (HaCaT) cell line by TaqMan Low Density Array and Western blot. Functional analyses such as cell division and apoptosis were also performed. We found that treatment with ZnCl2 did not increased the cell division of keratinocytes significantly, and did not affect the UVB induced cell division block, however, significantly reduced the proportion of apoptotic cells and significantly enhanced the number of necrotic cells at 24 hour after UVB irradiation, while the proportion of survival cells was not changed. Besides of an increase in the transcription of zinc homeostatic genes (metallothionein isoforms, zinc transporter), expression of the anti-inflammatory heme oxygenase-1 gene was also up-regulated following ZnCl2 treatment. UVB induced down-regulation of some zinc homeostatic genes (MT1E, MT1F and immune-modulating TRIM26). Interestingly, zinc set back the down-regulating effect of UVB on the expression of MT1E and apoptotic HIPK2 genes, and enhanced the Bcl2 down-regulation in a synergistic manner. It is assumed that zinc has an immune-modulating role in skin UVB response.

692

Triptolide, a diterpenoid triepoxide, prevents ultraviolet B-induced sebum production and hyperkeratinization in hamster skin *in vivo* and *in vitro*

A Ito, N Akimoto and T Sato *Department of Biochemistry and Molecular Biology, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan*

Sebaceous glands are an important skin appendage to maintain the cutaneous barrier by sebum secretion. Ultraviolet B (UVB) irradiation of the skin is considered to interrupt the biological function(s) of the cutaneous barrier by inducing excessive production and secretion of sebum and hyperkeratinization and acute inflammation in the epidermis. Since extracts of natural products including Chinese herbs have been reported to exert beneficial actions for the treatment of photoaging, there are few candidates to improve sebum production and secretion in UVB-irradiated skin. In this study, to develop an anti-photoaging agent(s), which may prevent the augmentation of sebum production and epidermal thickness, we examined the effect of triptolide, a diterpenoid triepoxide from *Tripterygium wilfordii* Hook. f. on sebum production, secretion, and dyskeratosis in UVB-irradiated hamster skin. UVB irradiation augmented both the biosynthesis and secretion of sebum in hamster sebaceous glands *in vivo* and *in vitro*. Triptolide was found to inhibit the UVB-augmented production of triacylglycerol (TG), a major component of sebum, by decreasing the activity of diacylglycerol acyltransferase, a rate-limiting enzyme of TG biosynthesis in hamster sebocytes. In addition, triptolide transiently enhanced the excretion of intracellularly accumulated sebum, and thereafter to exhaust intracellular sebum in the sebocytes. Furthermore, triptolide slightly prevented the UVB-induced epidermal thickness in the skin of hamsters. These results provide novel evidence that triptolide is a novel anti-photoaging agent by inhibiting not only excess production of sebum in sebaceous glands but also hyperkeratinization in the epidermis of UVB-irradiated skin, in addition to its known anti-oxidative and -inflammatory actions.

693

SNEV haploinsufficiency accelerates premature skin aging in response to 8-methoxypsoralen/UVA treatment in mice

R Monteforte,[1] F Gruber,[2] E Tschachler[2] and J Grillari[1] *1 Department of Biotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria and 2 Department of Dermatology, Medical University of Vienna, Vienna, Austria*

PUVA (8-methoxypsoralen/UVA) treatment is widely used for skin diseases like psoriasis. The therapeutic effect depends on inducing DNA damage in form of interstrand cross links. One prominent side effect of repeated PUVA treatment is premature aging of the skin. The protein SNEV (Senescence Evasion Factor) is a nuclear matrix protein involved in diverse pathways, among them DNA repair. Our aim is to investigate the involvement of SNEV in the DNA damage response following PUVA treatment with particular interest in understanding the role of SNEV in the mechanism at the basis of premature aging. We performed PUVA treatment on the skin of SNEV haploinsufficient (SNEV+/-) (SNEV knock out is embryonic lethal) and wild type (WT) mice. We treated the back skin of young SNEV+/- and WT mice with 8-MOP (8-methoxypsoralen; 1mg/mL) and 10J/cm2 UVA and analyzed the skin 48 hours after treatment. SNEV basal level in SNEV+/- mice is 3 folds lower than in WT mice. After PUVA treatment, SNEV level increased of 2 folds in both SNEV+/- and WT mice. The increase of SNEV was concomitant with the increase of γ- H2AX (known DNA damage marker). Then, we treated the back skin of old SNEV+/- and WT mice with 8-MOP (0.1mg/mL) and 1J/cm2 UVA three times a week for 2 weeks. At the end of the treatment, we kept one PUVA treated group including SNEV+/- and WT mice for 1 month in order to evaluate signs of premature aging. SNEV protein level decrease with age. In SNEV+/- old mice SNEV level is barely detectable. SNEV basal level in WT old mice is 3 folds lower than in WT young mice. The skin of SNEV+/- mice 1 month after the PUVA treatment exhibited epidermal thinning (a characteristic sign of aged skin) with an epidermis thickness 2 folds lower than in WT mice. Our data indicate that SNEV plays a role in the response to DNA damage following PUVA treatment and it is important to prevent premature aging of the skin.

694

In-vitro photodynamic activity of two zinc and aluminium sulphonated phtalocyanines

M Tampa, C Matei and R Ion *"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

Photodynamic therapy (PDT) is a treatment method implying the simultaneous presence of a light source, molecular oxygen and a photosensitiser selectively retained in the tumor cells; phtalocyanines are chemical compounds related to porphyrins, consequently possessing the theoretical potential of acting as good photosensitisers. Our study aims to assess the changes in cellular viability and proliferative capacity induced by photodynamic therapy using two different phtalocyanine derivatives, namely zinc tetrasulphonated phtalocyanine (ZnS4Pc) and aluminium di-sulphonated phtalocyanine (AlS2Pc) in dysplastic keratinocytes. Keratinocytes from standardised human dysplastic oral keratinocyte (DOK) cell line have been incubated with increasing concentrations of ZnS4Pc and AlS2Pc in aqueous solutions and then irradiated with red light PDT lamp at 632 nm, at a fluence rate of 30 mW/cm2, for 30 minutes. The cell viability before and after irradiation was assessed using trypan blue exclusion test and extracellular LDH release test with Cytotox96 non-radioactive cytotoxicity assay; the proliferative capacity was evaluated by a colorimetric method based on the conversion of tetrazolium salt into formazan measuring absorbance at a wavelength of 490 nm in samples before and after PDT lamp exposure. Apoptosis was assessed using annexin V-FITC and propidium-iodide method. Following PDT irradiation, apoptosis was induced by both studied compounds. At similar concentrations below dark-toxicity level, AlS2Pc proved to be more efficient than ZnS4Pc in terms of reducing cell viability and proliferation. The most efficient concentrations were 7.6 micrograms/ml and 4,36 micrograms/ml for AlS2Pc and ZnS4Pc respectively. The cytotoxic effects positively correlated with the photosensitiser concentration ($r=0.95$, $p<0.027$ for ZnS4Pc, $r=0.94$, $p<0.036$ for AlS2Pc). The study proves that the two tested phtalocyanine derivatives possess a good potential for use as photosensitisers in PDT. AlS2Pc proves to be a more effective photosensitiser than ZnS4Pc in PDT on cultured dysplastic keratinocytes.

695

Photo-ageing repairing mechanisms after UVA damage on FT-skin Marisa Meloni,* Barbara De Servi*, G. Baratto° and A. Semenzato° *VitroScreen, Via Mosè Bianchi, 103 20149 Milan °UNIR&D, Padua

M Meloni,[1] B De Servi,[1] G Baratto[2] and A Semenzato[2] *1 VitroScreen, Milano, Italy and 2 UNIR&D, Padova, Italy*

UVA radiation adversely affects skin health and appearance: a better understanding of early molecular pathways inducing long-term effects as photoageing can be reached by experimental in vitro models, being limited, for ethical concerns the exposure of human volunteers to UVA. In this study by using an in vitro skin equivalent, the dynamic of the genomic response after acute UVA exposure has been monitored during the post-irradiation periods of 6h, 24h and 7 days. The dose of 12 J/cm2, has been emitted by a solar simulator (Oriel 1Kw) as UVA source, and confirmed as relevant in inducing gene expression modifications of the selected biomarkers (COL1A1, DCN, ELN, FBN1, IL-1α, MMP1 and GPX and GSR only at 6h) at mRNA level by qRT-PCR. The transcriptional study has been conducted with 2 antioxidant molecules (2-oxo-1,3,thiazolidine and carcinine chlorohydrate) and with different sun products: SPF values 10 and 50 in order to modelize the pure UV filters effects. Tissue response in absence of treatment (positive control) was in agreement with previously published data and consist in a down regulation of ELN, FBN1, DCN, COL1A1) and in an over-expression of MMP1 and IL-1α. The UV filters efficacy has been established in term of inflammation reduction (MMP1 and IL-1α) and collagen network protection (COL1A1, FBN1). The 2 antioxidant molecules have shown a different mechanism: after 24h they were both able to activate a defensive response against elastin's damage but in the long term read-out (7days) they acts differently: the 2-oxo-1,3,thiazolidine by strongly reducing the inflammatory response (down regulation of MMP-1) and by activating the adaptive response (over-expression of DCN); the carcinine chlorohydrate activates the adaptive response. The transcriptomic approach applied to 3D human tissues appears to be an encouraging method for gaining a deeper understanding of the UVA effects on skin and for studying the dermal response with non-invasive, ethical and quantitative techniques.

696

Tocopherols and tocotrienols (Toco 3.0) reduce proinflammatory interleukines expression of HaCaT keratinocytes after UVB irradiation

M Mattii,[1] S Lembo,[1] R Di Caprio,[1] A Balato,[1] S Manfredini[2] and G Monfrecola[1] *1 Dermatology, University Federico II, Naples, Italy and 2 Pharmaceutical Science, University of Ferrara, Ferrara, Italy*

Ultraviolet radiation (UV) has profound effects on human skin, causing sunburn, inflammation, cellular-tissue injury, cell death, skin cancer and, most of them, are mediated by several keratinocytes produced cytokines. Tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ), also named "tocols", are natural compounds found in the Vitamin E complex and their antioxidant behaviour is well-established. Moreover, tocopherols play an important role in skin homeostasis, limiting trans-epidermal permeability of liposoluble substances and regulating keratinocytes turn-over. Recently, it has been demonstrated that tocols are able to reduce gene expression of cyclooxgenase-2 (COX-2), interleukin (IL)-1β, IL-6, andMCP-1 of UVB irradiated keratinocytes. In the present study we wanted to investigate whether tocols could be able to modulate the expression of IL-8 and TNF-α in UV-irradiated keratinocytes. Both, UVB dose and suitable tocols concentrations, have been selected after multiple cell viability experiments. HaCaT cells were irradiated with UVB (100 mJ/cm°) in the presence or absence of single or mixed tocols and cytokine mRNA levels were examined, by quantitative reverse polymerase chain reaction, 6 or 24 hours post irradiation, depending on the specific release peak of each cytokine. Our results showed that either single or mixed tocols significantly down-regulated IL-8 and TNF-α gene expression. Since the regulation of immunomodulatory cytokines is considered an essential part of treatment in multiple inflammatory diseases, tocols may be an interesting option to improve or prevent UV-induced or aggravated clinical conditions.

697

The Effects of Infrared Radiation versus Ultraviolet Radiation in Human Skin Cells

JA Latimer, EV Russell and MA Birch-Machin *Dermatology, Newcastle University, Newcastle, United Kingdom*

It is well known that solar radiation can cause damage to human skin which can physically be observed in cases of photoageing. Substantial research has found ultraviolet radiation (UVR) to be a major cause of photoageing as well as photocarcinogenesis. However, of the solar radiation which reaches human skin, only 7% is made up of UVR in contrast to ~54% of infrared radiation (IRR). Accordingly it has been reported that IRR may contribute to the damage solar radiation creates in human skin, yet recent investigations into the effects of IRR on human skin are not entirely clear. The aim of this study was therefore to compare specific IRR induced effects with those which are well known to be caused by UVR. Cells were irradiated with a maximum dose of either ~570 J/cm2 IRA (hydrosun 750) or ~10 J/cm2 UVA (glass filtered Philips TL 20W/09 RS) and ROS production was measured using the ROS detecting fluorescent probes DHR123 and DCFDA and fluorimetry (± SD, n=8). This maximum dose of UVA caused a significant 200 fold and 2.2 fold increase in fluorescence in the presence of both of the ROS detecting fluorescent probes, DHR123 ($P<0.0001$) and DCFDA ($P<0.05$) respectively. There was no significant difference seen between the un irradiated and IRA irradiated cells when DHR123 was used, there was however a significant ($P<0.05$) 2.6 fold increase in ROS generation detected in response to the maximum IRA dose when using DCFDA. DHR123 is a non-specific indicator of peryoxynitrite; therefore this data may suggest subtle differences between IRA and UVAs abilities to produce peryoxynitrite. Interestingly this dose of IRA caused no damage within mitochondrial DNA when assessed using a long PCR method that examines the majority of the genome. This work provides an ongoing platform to investigate the effects of antioxidants and to elucidate the mechanism of the reported IRR induced effects.

698

Systemic photoimmunoprotection: could a Polypodium Leucotomos extract enhance the activity of Photo Dynamic therapy on scalp Actinic keratosis

M Auriemma, G Febbo, M Tracanna, C Ruggiero and P Amerio *Dermatologic Department, G. d'Annunzio University, Chieti, Italy*

Actinic keratosis is a sun-induced neoplasm that affects the epidermis and which could evolve into squamous cell carcinoma (SCC). Over 80% of the lesions are found in sun-exposed areas such as scalp, face, neck and limbs. Photodynamic therapy PDT is a non-invasive therapeutic method that uses the interaction between visible light and a sensitizing agent (Protoporfirin IX) to generate cell death. PDT is indicated for various dermatological diseases: non melanoma skin cancers (NMSC) and preneoplastic keratinocytic lesions. Polypodium leucotomos (PLE) is a tropical plant part of the Polypodiaceae family used in the ethnical medicine for inflammatory skin conditions. The rizoma, the active part of the plant, is contains phenolic compounds with antioxidant antinflammatory and immunomodulatory properties. An extract of the plant is commercially available as oral supplementation (PLE). Twelve (12) patients suffering of scalp Actinic keratosis (AKs) were enrolled. The study population was divided into two groups: six (6) received. PLE supplementation soon after PDT treatment and six (6) received only PDT treatment. The total count of scalp AKs was recorded before PDT treatment (T0) and after 2 (T1) and six (6) months (T2) from treatment. The PDT treatment consisted in 2 different sessions (one week apart) of metil-amino-levulinic acid application and irradiation with a led light 630nm device. Al treatments and measurements were performed in winter-time. Both PDT and PDT + PLE treatments schedule resulted in a reduction in AKs count. However, PDT + PLE determined a major decrease in AKs count when compared to PDT treatment alone PDT + PLE = -57,3% at T1; -75% at T2; while PDT alone AKs reduction is -51,5% at T1 and -57,57% at T2). Our preliminary data suggest that Polypodium Leuotomos Extracts oral supplementation in association with PDT may enhance PDT efficacy in the treatment of AKs thus reducing the needs of repeated PDT treatments.

699

Effect of long term PUVA treatment on photoaging in mice with epidermal MnSOD2 deficiency

A Bánvölgyi,[1] N Gyöngyösi,[1] D Haluszka,[1] M Wlaschek,[2] K Scharffetter-Kochanek[2] and N Wikonkal[1] *1 Department of Dermatology, Venerology and Dermatooncology, Semmelweis University, Budapest, Hungary and 2 Department of Dermatology and Allergic Diseases, Ulm University, Ulm, Germany*

The manganese superoxide dismutase 2 (MnSOD2) is a key protein in cells to eliminate free radicals. It's partial or complete lack in individual organs leads to early aging and loss of certain functions. This study investigates the effect of chronic UVA irradiation on photoaging delivered to epidermal homozygous MnSOD2 knockout mice. We used mice with normal MnSOD2 activity as a control group. A daily dose of 2-3 J/m2 UVA radiation was given to animals three times a week after the use of psoralen as topical photosensitizer. We evaluated the degree of photoaging by measuring the thickness of the epidermis in tissue samples taken from various body parts that were exposed to different levels of radiation. We also measured thickness and the consistency of the skin wrinkles on the head of the mice, as a steady point, using a score system developed by us, based on the data above. Furthermore, we stain tissue samples with connective tissue selective staining method that showed the degeneration and fragmentation of the collagen and elastin system, as a sign of photoaging. The data showed that the extent of wrinkling and other alterations due to photoaging was more extensive in knockout animals. The analysis proved that the MnSOD2 has key role in the cells' free-radicals-elimination system. In case MnSOD2 is not present, more free radicals develop cause early skin-aging.

700

Impact Assessment of Energy Saving Lamps on Photosensitive Skin

L Fenton, J Ferguson, S Ibbotson and H Moseley *Photobiology Unit, University of Dundee, Dundee, United Kingdom*

The purpose of the study was to determine whether energy-saving lamps aggravate photosensitive skin, and to provide evidence based guidelines which help minimise ultra-violet risk. The skin of patients, with a wide range of photosensitive conditions, was exposed to emissions from a single enveloped compact fluorescent lamp, a double enveloped compact fluorescent lamp and a light emitting diode. The lamps were positioned at a worst-case scenario distance of 5cm, using a four aperture minimal erythemal dose template. Observation of any erythemal responses was noted immediately, 30mins, 7 hours, 24hours and 48hours post irradiation. The single enveloped fluorescent lamp induced erythemal responses in 31 out of 195 patients. 10 of these patients, where erythema was induced with the single enveloped lamp, were further tested with a double enveloped compact fluorescent lamp. In 6 of these patients no erythema was induced, the further 4 patients had responses that were reduced in erythemal grading. The light emitting diode did not induce skin erythema in any photosensitive patients. Individuals with Chronic Actinic Dermatitis are the most at-risk patient group from ultra-violet emissions from compact fluorescent lamps. Whilst double enveloped compact fluorescent lamps are a safer alternative for photosensitive individuals, the ultraviolet present may still aggravate the skin of extremely sensitive patients. Light emitting diodes provide a safer alternative energy-saving lamp that reduces the risk of aggravating ultra-violet sensitive skin.

701

Beneficial Effect of Coumestrol on Ultraviolet B-Induced Skin Photoaging through Mitochondrial Biogenesis

S Kim, J Kim, D Seo and S Lee *Beauty Food Research Institute, R&D Center, Amorepacific Corporation, Yongin-si, Giheung-gu, Republic of Korea*

Coumestrol is one of phytoalexins synthesized in response to environmental stress, and commonly found in natural foods such as alfalfa sprouts, clovers, and soybean. In the present study, we investigated the mechanism underlying protective effect of coumestrol on UVB-induced photoaging in human dermal fibroblasts. We found that pretreatment with coumestrol enhanced the UVB-suppressed mitochondrial biogenesis through regulation of Sirt1 expression and activity, and its downstream gene regulation such as PGC-1α, NRF1, and TFAM. Moreover, the ATP and ROS production was restored to normal and the formation of advanced glycation endproducts leading to skin photoaging in skin fibroblasts was blocked by coumestrol pretreatment before UVB irradiation. These findings indicate that coumestrol might potentially protect skin photoaging induced by mitochondrial damage and glycated protein production in dermal fibroblasts.

702

Modulation of Pro-Inflammatory Cytokines Response in Keratinocytes and Fibroblasts Culture Exposed to UVA or UVA Radiation

K Isabelle, J Valentin, J Olivier, K Robin, S Sylvianne and P Eric *Life Sciences Department, LVMH recherche, Saint Jean de Braye, France*

Ultraviolet (UV) radiations are involved in skin ageing due to photo-damage and, particularly, to oxidative stress. About 15% of UVB reach the basal layer of epidermis while 50% of UVA penetrate more deeply until the dermis. Keratinocytes and fibroblasts play a key role in the induction of inflammatory response. The study was to investigate the effects of UV radiations on these two major skin cells. More precisely, the impact of UVA & B on pro-inflammatory cytokines production (IL-b, IL-6 and IL-8) was evaluated. Normal Human Keratinocytes (NHEK) and Normal Human Fibroblasts (NHDF) were seeded in a 6 well-plate (100.000 cells/mL). At confluence, the cells were irradiated with 50 mJ/cm2 UVB or 4 J/cm2 UVA. After 24 hour treatment, the cell culture supernatants were harvested. After checking the cell cytotoxicity (XTT), IL-1b, IL-6, and IL-8 cytokines from supernatants were measured by xMAP technology (Millipore). Our results showed that, after UVB radiation, IL-6 concentration was 36.74 pg/ml (1400% of control) and 104.60 pg/ml (5800% of control) for NHDF and NHEK, respectively. In contrast, the UVA radiation was less effective on IL-6 production (814% for NHDF and 145% for NHEK). The IL-8 amount from UVB-irradiated NHDF was 72.76 pg/ml (245% of control) while its level dramatically increased (522.42 pg/ml) in UVB-irradiated NHEK. From UVA-irradiated NHDF and NHEK, the increase of IL-8 level from control is closed to 150%. In our experimental conditions, NHDF did not constitutively secrete IL-1b and no production was observed whatever the UV radiation treatment. However, the UVB radiation of NHEK highly induced IL-1b secretion (2000% of control), compared to the UVA radiation (130% of control). The production of pro-inflammatory cytokines depends on the skin cells and ultraviolet (UV) radiation wavelengths. These first results showed the importance of the design of the investigational model (cells and radiation treatment) to identify new anti-inflammatory compounds.

703

UVR induced stress on patients with Porphyria Cutanea Tarda

G Smaranda Rodica,[1] S Caius[2] and G Smaranda Laura[1] *1 Physiology, University of medicine and Pharmacy "V. Babes", Timisoara, Romania and 2 Dermatology, University of medicine and Pharmacy "V. Babes", Timisoara, Romania*

Reactive oxygen species (ROS) can induce tegument lesions. Ultraviolet radiation (UVR) is a major exogenous factor inducing ROS which reinforce cutaneous lesions in porphyria cutanea tarda (PCT) patients. In the present study we performed in vitro investigation of some aspects of the oxidative stress in PCT patients as well as the UVR-induced production of ROS. UVR activates the oxidative metabolism and production of ROS, and we developed an in vitro model based on this finding. Venous blood on heparin was taken from 15 PCT-patients treated in the Hospital for Dermato-venerology of Timisoara city (Romania). It was divided in three series of samples that were irradiated for 5, 10, and 15 min. The UVR exposure was performed in a dark room by means of an 80 W biological quartz lamp placed at a distance of 20 cm from the sample. The phagocytotic capacity was estimated by nitrobluetetrazolium (NBT) reduction test that indirectly indicated the activation of oxidative metabolism and ROS production during phagocytosis. In each series the pre-irradiation state served as the control. Hematocrit was determined before and after each UVR blood exposure. In the PCT patients the exposure time-dependent progressive increase of the NBT reduction (from 3.1±2.01% to 7.33±1.31%) indicated strong UVR-induced stimulating effect on the oxidative metabolism and ROS production of the granulocytes. Hematocrit progressive decreased with 5.66%, 9.43% and 18.87%, indicating that the UVR blood exposure induced the production of ROS also in erythrocytes. ROS caused lesions of the proteins and erythrocyte membrane enzymes, making the cells more fragile. The macroscopic hemolysis and hematocrit decrease can be explained by enhanced fragility of the erythrocytes after UVR exposure. These results obtained in vitro were correlated with the clinical database which showed aggravation of the cutaneous lesions after solar exposure. In conclusion ROS production, at least partially, contributes to the detrimental effect of UVR on the skin of PCT patients.