1227 Matrix Metalloproteinase (MMP) 12 is a UVA-specific biomarker
A. Toujas,1,2 D. Dalila,1 K. Goy,1 S. Sarkany,1 and A. Young2 1 St John’s Institute of Dermatology, King’s College, London, United Kingdom and 2 Photokrmatology, St Johns Institute of Dermatology, St Thomas’ Hospital, SE1 7HT, United Kingdom

The carboxy-terminal potential of UV (340-400 nm) is increasingly recognized as evidence accumu-
lates of its ability to induce cyclobutane pyrimidine dimers (CPD) ex vivo and in vivo in humans
which if unrepaired may lead to skin cancer. Despite widespread use in phototherapy, tanning lamps
and its abundance in terrestrial UVB, we lack data on its effects on gene expression in human skin.
Using ethylenediamine equivalent doses of UV-A and UV-B (0.38 cm2 for UVA and 0.32 cm2 for UVB
-1.4MD, minimal erythema dose), we assessed gene expression and protein changes in 12 skin types
of 11 individuals. The major upregulated pathway at 24h post UVA and UVB is extracellular matrix
remodeling ECM, but key spectral differences were demonstrated by RT-PCR for mRNA expres-
sion. It was more effective for MMP (p=0.0062), MMP3 (p=0.0016), MMP9 (p=0.028). There
is more MMP protein and activity in the epidermis in all volunteers. UVA and UVB upreg-
ulate MMP1 which is known to be induced by UVB, ROS, and via mediators such as TNF-α.
However UV does not form MMP12 mRNA suggesting that it is likely formed by an alternative
route to MMP1. Our data suggest that MMP12 is a UVA specific epidermal biomarker and we hypoth-
esize a role for ROS, possibly via oxidative damage to DNA (8oxodG) which has recently been
shown to be important in a melanoma mouse model. This is the first study to dissect spectral
deficiencies in ECM genes which may play an important role in photocarcinogenesis and photoaging.

1228 Keratinocyte-specific deletion in mice reveals gene dosage-dependent role for SIRT1 in UVB-
induced skin tumorigenesis
M. Ming,1 K. Soffani,1 CR Sheh,1 X Li1 and Y. Le1 1 Dermatology/Medicine, University of Chicago, Chicago, IL and 2 Laboratory of Signal Transduction, NIEHS/NIH, Research Triangle Park, NC 27709

The protein deacetylase SIRT1, a mammalian counterpart of the yeast silent information regulator
2 (Sir2), is a proto-oncogene of the sir2 family, regulates various pathways in metabolism, DNA
repair, and cell survival. However, the role of SIRT1 in cancer is still under debate. Here we show
that the role of SIRT1 in skin cancer development induced by the human skin carcinogen UVB
radiation is dependent on gene dosage. Keratinocyte-specific heterozygous deletion of SIRT1 increases
UVB-induced skin tumorigenesis, whereas homozygous deletion of SIRT1 decreases skin tumori-
genesis. In mouse skin, SIRT1 is haploinsufficient for UVB-induced DNA damage repair and expres-
sion and senescence pigmentum C. XPC. A protein critical for repairing UVB-induced DNA dam-
age. Similar to homozygous SIRT1 deletion, heterozygous SIRT1 deletion reduces XPC protein
levels and UVB-induced DNA damage repair. As compared with normal human skin, down-regulation
of SIRT1 is in parallel with down-regulation of XPC in human cutaneous squamous cell carcinomas
at both the protein and mRNA levels. In contrast, homozygous SIRT1 deletion in mouse skin augments
p53 acetylation and expression of its transscriptional target Nias, and sensitizes the epidermis to
UVB-induced apoptosis in vivo, while heterozygous SIRT1 deletion did not affect apoptosis. Ability of mice
with homozygous SIRT1 deletion do not develop tumors, these mice suffer severe solar injury. The
gene dosage-dependent function of SIRT1 in DNA repair and cell survival is consistent with the
opposing roles of SIRT1 in UVB-induced skin tumorigenesis and injury. Taken together, our results
indicate that, depending on the SIRT1 level, SIRT1 acts as a tumor suppressor and as an oncogene,
and they suggest an essential role for SIRT1 in skin homeostasis.

1229 Astaxanthin, a xanthophyll carotenoid, inhibits ultraviolet-induced apoptosis in keratinocytes
Y. Yoshida,1 and T. Shizuma Dermatology, University of Toyama, Toyama, Japan

Intracellular reactive nitrogen/oxygen species and apoptosis play important roles in ultraviolet (UV-
induced inflammatory responses in the skin. Astaxanthin (AST), a xanthophyll carotenoid, is a
nutrient with unique cell membrane actions and diverse clinical benefits. We herein investigated
the effects of AST on UV-induced apoptosis and the pro-inflammation cytokine expression in HaCaT
keratinocytes. AST (5 µM) caused a significant decrease in the protein content and mRNA levels of
iNOS and COX-2, and decreased the release of prostaglandin E2 by HaCaT cells after UVB (20
mJ/cm2) or UVC (5 mJ/cm2) irradiation. A marked increase in O2•− and H2O2 production was observed
in UV-treated HaCaT cells, while a decrease in CO2 and H2O2 production was not observed in AST-
-treated cells. Pretreatment of the cells with AST caused a significant inhibition of UVB- and UVC-
induced apoptosis, as shown by a DNA fragmentation assay. Furthermore, we found that the treat-
ment with AST caused a reduction in the UVB or UV-induced protein and mRNA levels of NF-
κB, IL-1α, and IL-1β in HaCaT cells. These results suggest that astaxanthin may be a protective agent
against UV-induced inflammation by decreasing the iNOS and COX-2 and inhibiting the apoptosis of keratinocytes.

1230 Characterisation of skin autofluorescence to establish its role in distinguishing between nor-
mal and diseased skin
A. de Barros,1 KS Robinson,1 H Mouldes,1 J Gardner2 and SH Bithell1 1 Photobiology Unit, University of Dundee, Dundee, United Kingdom and 2 School of Medicine, University of Dundee, Dundee, United Kingdom

Recent studies have highlighted the use of skin autofluorescence in the assessment of skin.
Intracellular reactive nitrogen/oxygen species and apoptosis play important roles in ultraviolet (UV-
induced inflammatory responses in the skin. Astaxanthin (AST), a xanthophyll carotenoid, is a
nutrient with unique cell membrane actions and diverse clinical benefits. We herein investigated
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against UV-induced inflammation by decreasing the iNOS and COX-2 and inhibiting the apoptosis of keratinocytes.

1231 UVA induces the aging-associated progerin through formation of oxidative damage and sub-
sequent alternative splicing of LMNA
H. Takeuchi,1 H. Dafou,1 KG Rys,1 R Sarkany1,2 and A Young1 1 Photobiology Unit, University of Dundee, Dundee, United Kingdom and 2 St Johns Institute of Dermatology, King’s College, London, United Kingdom

The carcinogenic potential of UVA (340-400nm) is increasingly recognized as evidence accumu-
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deficiencies in ECM genes which may play an important role in photocarcinogenesis and photoaging.

1232 Prostaglandin E2-EPA signaling mediates the development of a newly established niacin defi-
cency-induced pelagra model
K. Sugita,1 T. Nomura,1 M. Nakamura,1 Y. Yikasa1 and K Kabashima1 1 Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu, Japan and 2 Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan and 3 Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan

Pelagra, which is characterized by the four Ds of dermatitis, dermatitis, dermatitis, and death, is
a chronic wasting disorder that results from a marked cellular deficiency of niacin. Although pella-
gra continues to be a significant health issue worldwide, the molecular mechanisms underlying its
hallmark abnormalities, including photosensitivity dermatitis, are unclear. Here, we show that
enhanced photosensitivity in mice was induced by treatment with a niacin antagonist as well as
niacin-deficient diets, and that severe diarrhea with weight loss was induced by niacin deficiency,
both of which suggest that this is a good model of pelagra. Intriguingly, niacin deficiency induced
expressions of COX-2 and prostaglandin E2 synthase (PGS) mRNAs. The niacin defici-
cency-induced photosensitivity was alleviated by a COX inhibitor, knockout of Pgs genes, or block-
ade of E2 receptor signaling by a specific E2 antagonist. In line with the above murine findings,
single-dose s.c. and p.o. treatment with niacin-deficient diets markedly increased the expression of PGS
mRNAs in keratinocytes at the sun-exposed area. Taken together, these findings indicate that niacin defici-
cy results in the development of photosensitivity in pelagra that is mediated by signaling through
prostaglandin and cyclooxygenase. The blockade of PG2-E2 signaling may be a new strategy in the prevention and treatment of pelagra.
1233 Impact assessment of energy efficient lamps on individuals with lupus erythematosus versus healthy individuals

J Ferguson,1 SH Hbition,1 RS Dawe,1 S Sibum2 and H Moseley1 1Photobiology Unit, University of Dundee, Dundee, United Kingdom and 2Photobiology Research & Innovation, L’Oréal, Clichy, France

To assess if different types of energy efficient lamps can exacerbate photosensitive lupus erythematosus (LE). 10 patients with LE and 5 healthy volunteers were exposed to emissions from an energy efficient halogen (HE), compact fluorescent lamp (CFL) and a light emitting diode (LED) individually on three consecutive days in order to assess skin response. The photoprovocation tests were examined for up to 3 weeks post irradiation. Delayed skin erythema was induced by the CFL in 6 LE patients and 1 healthy subject. The erythema induced by the CFL in 2 of the LE patients was persistent for 3 weeks. One LE patient produced delayed skin erythema from the HE emissions, all healthy subjects had negative responses. One LE patient produced abnormal immediate erythema responses to all of the light sources; this patient was shown to have abnormal urticarial responses to UVA radiation. All other LE patients and the healthy subjects produced normal responses to the LED. This study shows that CFLs have the potential to exacerbate photosensitive LE. CFLs can also induce erythema in healthy individuals. LEDs provide a safer alternative light source without the UV risk.

1235 UVR-induced DNA damage in melanocytes is dependent upon skin constitutive pigmentation

S Del Bino, J Sok and F Berend Research & Innovation, L’Oréal, Clichy, France

Melanin amount inversely correlates with the risk of skin cancer, including melanoma. However data on the specific UVR impact on melanocytes in skin of different constitutive pigmentation are scarce. The present study analyzed UVR-induced DNA damage within melanocytes from different skin color types. Samples from subjects objectively classified into Light, Intermediate, Tan, Brown and Dark according to their Individual Typology Angle (ITA) based on colorimetric parameters, were exposed to increasing doses of Solar Simulated Radiation (SSR). Detection of DNA damage occurring specifically in melanocytes was performed using a double staining for cycloubutane thymine dimers (CPD) and 8-oxo-deoxyguanosine (8-oxoG). The major UVR-induced DNA photoproduct and Tyrosinase related protein 1 (TRP1), a key enzyme in melanin synthesis. Our results showed accumulation of CPD in melanocytes detected at the lowest dose, with a steep increase with dose, in Light, Intermediate and Tan skin. Additionally, 8-oxoG equivalent doses (based also on sunburn cell formation), ~ 80-100% of melanocytes were CPD-positive in Tan to Light skin types. In contrast, in Dark and Brown skin types, CPD were only found in ~15% of melanocytes at the highest dose. This study shows a relationship between UVR impact on melanocytes and skin colour type, according to the ITA value. These differences support the superior protective role of melanin in very highly pigmented skin types where total melanin content, especially eumelanin, is higher. Even more interestingly, our results show that enzymatic free radical does not induce comparable DNA damage in melanocytes. Lessons in melanocytes may explain the higher risk of lighter skin types at developing melanoma. They also reveal that DNA of melanocytes from Tan skin, although corresponding to a pigmented phenotype, is actually damaged by UVR. This indicates that photoprotection should not be limited to the very light skin types, but extended to moderately pigmented skins where a prevalence of pigmentary disorders is clinically observed.

1236 Photodynamic therapy (PDT) downregulates the function of regulatory T cells in patients with esophageal squamous cell carcinoma

F Meloni,1 J Lindemann,2 J Défise,1 J Sok and F Bernerd 1Photobiology Research & Innovation, L’Oréal, Clichy, France and 2Dermatology Department, Hôpital LArche, Nice, France

Photodynamic therapy (PDT) constitutes the administration of a photosensitizer (PS) to the patient, followed by visible light delivery to the cancerous area. The light activation of the PS triggers a phototoxic reaction that culminates in the production of highly reactive single oxygen (1O2) and immediate cell damage. This causes apoptosis and necrosis of illuminated tumor cells, shuts down tumor microvessels, and induces an acute inflammatory response. The reports on the effect of PDT on immune function are controversial, depending on the type of immune response. Previous work in experimental models has revealed that depletion regulatory T cells (Tregs) may potentiate the efficacy of PDT. We therefore became interested to investigate the immunological changes induced by PDT and the effect of PDT on level and function of Tregs (CD4+CD25highCD127lowFoxP3+) in patients with squamous cell carcinoma (SCC). Such an effect may be crucial in PDT-treated patients with esophageal squamous cell carcinoma (SCC), in whom prolonged survival has been reported after multi-treatment approaches. To investigate the hypothesis blood was collected from patients with invasive ESCC before PDT and 7 and 14 days after treatment. Treg levels in the blood were quantified by FACS and Treg function by co-culture proliferation assays with effector cells. Our results indicate that PDT significantly diminishes by approximately 50% the suppressive capacity of peripheral Treg from ESCC patients whereas their Treg levels seem to be unaffected. This is important since FoxP3+ immunohistochemical staining of ESCC biopsies revealed massive infiltration by Tregs within tumor areas, compared to healthy esophageal mucosa. A better understanding of the immunological events linked to PDT is desirable to improve treatment strategies and the ultimate outcome in patients treated with cancer such as ESCC.

1237 Diversity of biological alterations induced by longwave UVA (UVA1) in human reconstructed skin

J Delvaglio, N Schweitz, J Defise, N Sandor, J Del Bino, F Bernerd and F Berend Research & Innovation, L’Oréal, Clichy, France

Solar UV exposure induces photaging and photocarcinogenesis. Among the UV range of wavelengths reaching the Earth ground, longwave UVA (340-400nm) can represent up to 80% of total UV. UVA1 show high penetration properties reaching the deep dermis. The negative role of total UV on skin aging is clinically observed. Our results related to PDT is desirable to improve treatment strategies and the ultimate outcome in patients treated with cancer such as ESCC.

1238 Actinic lentigines are characterized by a higher melanin content and a severe disorganization of the cutaneous architecture

J Defise,1 S Nuzzo,1 M Minot,1 P Bastien,2 E Warick,2 C Chapignon2 and J Ottone1 1Dermatology, and 2Dermatology Research Department, L’Oréal Research & Innovation, Clichy and 2Dermatology Department, Hôpital LArche, Nice, France

Actinic Lentigines (AL) are benign skin hyperpigmented lesions very frequently found in elderly people on sun-exposed sites. However, their aetiology and biological characteristics remain not well defined. The present study aimed at better describing morphological and biological features of AL expressing a similar clinical phenotype i.e. a so-called elongated/homogeneous pattern-detected by epiluminescence and quantified through a dedicated image analysis software. 15 Caucasian women (51-67 yrs, photos type II III) were included after clinical selection of AL on the dorsal side of their hands. Two skin biopsies were performed on each volunteer, one corresponding to the AL lesion and the other one at an adjacent non lesional skin area (NL). Each biopsy was processed under classic histology (HES, Fontana Masson) and immunostaining of melanogenic markers. HES staining showed that the high homogeneity of all lesions in term of structural organization and a drastic deformation of dermal-epidermal junction (DEJ) associated with epidermal invaginations within the dermal compartment. Morphometric analysis revealed in AL a significant increase in the basal layer thickness and a higher epidermal thickness. The evaluation of the number of melanocytes was included, in both basal and keratinocytes. These results give new insights into modifications of a large panel of biological functions affected by such exposure. They therefore strongly suggest the need for an adequate longwave UVA photoprotection.

1239 Development of an in vitro immune suppressive model

M Meloni, B de Servi, V Giammim and F Bernerd 1Photobiology Research & Innovation, L’Oréal, Clichy, Italy and 2Dermo-Cosmetic R&D, Ratiphamn,Madaus, Monza, Italy

Immuno-suppressive effects of ultraviolet radiation in humans include exacerbation of infectious diseases, skin cancer, and skin aging. As specific action level, UV induces the formation of immunosuppressive cytosine IL-10, responsible for a shift from Th1 to Th2 immune response involving the TNI-α. IL-10 promotes Th1 response establishing a protective mechanism against UV induced immune suppression. Aim of this study is to confirm the relevance of a reconstructed human epidermis (Episkin) as biological model for establishing an in vitro experimental UVA induced immune suppressive model. A sun simulator equipped with Xenon 1000 W, Hg 320 Short 1.3 mm, emitting UVA+UVB has been used to irraditiate the epidermal surface. A transrational study (IP-1PCR) based on IL-10, IL-12 and TNF-x gene expression has been performed to assess the tissue responses induced by UVA doses (0.5-3.2-4.4 MED) and recovery times (4,6,12,24h post irradiation) monitoring in parallel the cell viability (MTT test) and adenosine kinase release (Toxilight). IL-10 was not si-

deitically expressed after shorter recovery times (4 and 6h) indicating low immunosuppressive dam-
age; in the longer recovery times (16 and 24h) IL-10 up regulation revealed a strong immuno-
suppressive pathway. IL-12 expression was not significant for all the time points suggesting no interference in the immunosuppressive pathway and consistent with a physiological response in absence of immunosuppression. The results show that IL-12 can be used as biomarker of the cutaneous immune response downstream of the IL-10 induction pathway.

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Deficiency in N22 promotes UVA-induced expression of proinflammatory mediators in PUFA supplemented fibroblasts
Lyon, France

PUFA supplementation in human fibroblasts can increase UV sensitivity. We investigated the effects of PUFA supplementation on N22 skin fibroblasts for the first time. We found that PUFA supplemented fibroblasts were resistant to UVB radiation-induced apoptosis, but not against UVA. Additionally, we found that PUFA supplementation in N22 cells induced a significant increase in DNA damage caused by UVA, which is associated with an increase in Nrf2 expression. These results suggest that PUFA supplementation may have a protective effect against UVA-induced skin damage.

Deficiency in N22 promotes UVA-induced expression of proinflammatory mediators in PUFA supplemented fibroblasts.

Skin ageing is associated with oxidative stress and chronic inflammation even induced by repetitive UV exposure. In the presence of a prolonged condition of oxidative stress, the nuclear receptor PPARγ may play a role in the regulation of senescence-related processes. In this study, we investigated the effect of PPARγ agonism on senescence-associated biological markers in human keratinocytes (HaCaT cells) treated with UVA radiation. Our results showed that PPARγ agonists induced a significant increase in cell senescence markers, such as p16 and p21, and a decrease in cell viability. These findings suggest that PPARγ agonists may be useful in the treatment of skin ageing induced by UV radiation.

Prostaglandin E2 promotes UV radiation-induced immune suppression via DNA hypermethylation
F. Grueter, C. Mecking Ormelis, S. Karner, V.N. Bocchi, E. and T. Schachter
C. Institut Dermatologique, Medical University of Vienna, Vienna, Austria and 2 Department of Pharmacology and Toxicology, Medical University of Vienna, Vienna, Austria

Fibroblasts are important cells in the skin, as they produce collagen and other extracellular matrix components. In this study, we investigated the role of prostaglandin E2 (PGE2) in UV radiation-induced immune suppression in human dermal fibroblasts. We found that PGE2 inhibited UV-induced expression of proinflammatory cytokines, such as TNF-α and IL-6, and upregulated the expression of the anti-inflammatory cytokine IL-10. These results suggest that PGE2 may have a role in promoting immune suppression in the skin.

Inhibition of UV-induced immune suppression by 5-aza-dc in COX-2 deficient mice
S. Kamo,1 L. Duprat,1 T. Douki,2 H. Dromigny,1 B. Guiraud,1 S. Bessou-Touya1 and H. Duplan1
1 Institute for Environmental and Gender Biology, 2 National Centre for Scientific Research, Paris, France

The inhibition of UV-induced immune suppression by 5-aza-dc was evaluated in COX-2 deficient mice. The results showed that 5-aza-dc reversed the effect of PGE2 on UV-induced suppression of CHS response in COX-2 deficient mice. These findings uncover a previously unrecognized role of PGE2 in UV-induced suppression of CHS and that is mediated through epigenetic mechanism involving DNA methylation.

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ABSTRACTS | Photobiology

1245 Antagonizing effects and mechanisms of alzefin against UV-induced cell damage
S. Shyu,1 J. Jung,2 S. Kim,1 J. Lee3 and D. Park4 1 Experimental Research Team, Biospectrum, Inc, Seoul, Republic of Korea and 2 Department of Dermatological Health Management, Eulji University, Seoul, Republic of Korea Ultra violet (UV) radiation induces DNA damage, oxidative stress, and inflammatory processes in human keratinocytes, resulting in skin inflammation, photoaging, and photocarcinogenesis. Adequate protection against skin against the harmful effects of UV radiation is essential. Therefore, in this study, we investigated protective effects of alzefin, a yellow pigment of the flaxseed, against UV irradiation in human keratinocytes and epithelial equivalent models. Spectrophotometric measurements revealed that the alzefin extract and maxima were in the UVB and UVA range, and UV transmission below 376 nm was < 10%. Alzefin inhibited UV-induced cell death in human keratinocytes by inhibiting intrinsic apoptotic signaling. Alzefin also inhibited the UVB-mediated increase in lipid peroxidation and the formation of cyclobutane pyrimidine dimers. Furthermore, alzefin showed inhibitory effects on UVB-induced release of pro-inflammatory mediators such as interleukin-6, tumor necrosis factor-α, and prostaglandin-E2 in human keratinocytes by interfering with the PI3K/Akt and Raf/MEK/ERK unphosphorylation and of actin at Ser235/236 demonstrated strong phosphorylation throughout the entire epidermis following UV exposure. In conclusion, we showed that all three complexes enhanced keratinocytes viability after UVB exposure. More importantly, in vitro study showed that all three complexes enhanced keratinocytes viability after UVB exposure. The study results showed that protective effects of visible light on reducing sun-associated aging.

1246 Protective effect of tioproline and its enhancement by other active ingredients on UV-induced skin damage
N. Wang,1 N. Fernandez,2 J. Engel,1 N. Lang,1 M. Gallagher,3 DE Newby,2 M. Feilisch1 and RB Wellens1 1 Dermatology, University of Edinburgh, Edinburgh, United Kingdom, 2 Cardiology, University of Edinburgh, Edinburgh, United Kingdom and 3 Centre for Inflammation Research, University of Edinburgh, Edinburgh, United Kingdom Skin, the outermost barrier of the body, has to cope with all kinds of environmental and physical insults. One major external factor is ultraviolet radiation. The negative effects of unprotected skin exposure to UV can be seen in cutaneous modifications causing collagen fibers alterations, pigmentary disorders, various types of dermatosis and skin cancer. These modifications are in part due to the generation of ROS within tissues. Many active cosmetic ingredients are now available to prevent and counteract the effects of UV rays on skin cells. Present study was aimed to evaluate in vitro protective effects of three antioxidant complexes comprised of tioproline and one or two other compounds exhibiting antioxidant properties like flavonoids RA or S2 and amino acid TioT. Tioproline, known as an anti-aging diet supplement, has lipotrophic effects and protects heart, liver and kidney against fat accumulation. It seems to be interesting to check if combining tioproline with other compounds in complex ROS gives a synergistic effect on skin cells UV-protection. In vitro study showed that all three complexes enhanced keratinocytes viability after UVB exposure. Moreover antioxidant molecules but also can show some benefits on the skin and they contribute to a synergistic effect of skin cells UV-protection. In vivo study showed that all three complexes enhanced keratinocytes viability after UVB exposure. The study results showed that proline in a complex with antioxidant molecules seems to be promising for creating modern anti-aging cosmetics and skin care products.

1247 UVA lowers blood pressure and vasodilates the systemic arterial vasculature by mobilisation of cutaneous nitric oxide stores
D. Bishop-Cotter,1 A. Zheng,1 N. Lang,1 M. Gallagher,2 DE Newby,3 M. Feilisch1 and RB Wellens1 1 Dermatology, University of Edinburgh, Edinburgh, United Kingdom, 2 Cardiology, University of Edinburgh, Edinburgh, United Kingdom and 3 Centre for Inflammation Research, University of Edinburgh, Edinburgh, United Kingdom The incidence of hypertension and cardiovascular disease (CVD) correlates with latitude and rises below 376 nm was < 10%. Afzelin inhibited UVB-induced cell death in human keratinocytes by inhibiting intrinsic apoptotic signaling. Alzefin also inhibited the UVB-mediated increase in lipid peroxidation and the formation of cyclobutane pyrimidine dimers. Furthermore, alzefin showed inhibitory effects on UVB-induced release of pro-inflammatory mediators such as interleukin-6, tumor necrosis factor-α, and prostaglandin-E2 in human keratinocytes by interfering with the PI3K/Akt and Raf/MEK/ERK unphosphorylation and of actin at Ser235/236 demonstrated strong phosphorylation throughout the entire epidermis following UV exposure. In conclusion, we showed that all three complexes enhanced keratinocytes viability after UVB exposure. More importantly, in vitro study showed that all three complexes enhanced keratinocytes viability after UVB exposure. The study results showed that protective effects of visible light on reducing sun-associated aging.

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1249 Ultraviolet B irradiation increases phosphorylation of ribosomal protein S6 in human skin
N. Wang,1 and I. Healy2 1 Efficacy Research Team, Biospectrum, Inc, Seoul, Republic of Korea and 2 Department of Dermatology, Medical University of Tennessee Health Science Center, Memphis, TN Protective effect of tioproline and its enhancement by other active ingredients on UV-induced skin damage
N. Wang,1 N. Fernandez,2 J. Engel,1 N. Lang,1 M. Gallagher,3 DE Newby,2 M. Feilisch1 and RB Wellens1 1 Dermatology, University of Edinburgh, Edinburgh, United Kingdom, 2 Cardiology, University of Edinburgh, Edinburgh, United Kingdom and 3 Centre for Inflammation Research, University of Edinburgh, Edinburgh, United Kingdom Skin, the outermost barrier of the body, has to cope with all kinds of environmental and physical insults. One major external factor is ultraviolet radiation. The negative effects of unprotected skin exposure to UV can be seen in cutaneous modifications causing collagen fibers alterations, pigmentary disorders, various types of dermatosis and skin cancer. These modifications are in part due to the generation of ROS within tissues. Many active cosmetic ingredients are now available to prevent and counteract the effects of UV rays on skin cells. Present study was aimed to evaluate in vitro protective effects of three antioxidant complexes comprised of tioproline and one or two other compounds exhibiting antioxidant properties like flavonoids RA or S2 and amino acid TioT. Tioproline, known as an anti-aging diet supplement, has lipotrophic effects and protects heart, liver and kidney against fat accumulation. It seems to be interesting to check if combining tioproline with other compounds in complex ROS gives a synergistic effect on skin cells UV-protection. In vitro study showed that all three complexes enhanced keratinocytes viability after UVB exposure. Moreover antioxidant molecules but also can show some benefits on the skin and they contribute to a synergistic effect of skin cells UV-protection. In vivo study showed that all three complexes enhanced keratinocytes viability after UVB exposure. The study results showed that proline in a complex with antioxidant molecules seems to be promising for creating modern anti-aging cosmetics and skin care products.

1250 Ultraviolet B (UVB) radiation activates systemic hypothalamic-pituitary- adrenal (HPA) axis expression in C57BL/6 mice
E. Mikolajewski,1 K. Nejat,2 J. Bauman3,4, Z. Cooke4 and A. Smolinski1,5 1 Pathology and Laboratory Medicine, University of Tennessee Health Science Center, Memphis, TN and 2 Medicine, University of Tennessee Health Science Center, Memphis, TN The purpose of this project was to evaluate a role of UVB radiation in regulation of global homeostasis that is separate from the production of vitamin D3. Specifically, we tested the hypothesis that exposure of the skin to UVB will activate central HPA axis. The back skin of C57BL/6 female, 8 weeks old mice was shaved and irradiation anesthesia was applied. The mice with eyes covered by aluminum foil were irradiated with 400 μM/cm2 of UVB applied on shaved skin. After 12 and 24 h the mice were killed by cervical dislocation and plasma, skin, brain and adrenals were collected and extracted for RNA, proteins, peptides and steroids or fixed for immunohistochemistry (IHC). Levels of corticosterone-releasing hormone (CRH), uncorrected (U), β-endorphin (β-End) and aldehyde corticosterone (ACTH) and corticosterone (CORT) were measured by ELISA with precursor proteins detected by Western Blot (WB) and gene expression assessed by real-time PCR. β-End was used to evaluate in vivo expression of corresponding antigens. UVB stimulated plasma levels of CRH, U, β-End, ACTH and CORT. Furthermore, UVB irradiated skin expressed higher levels of URO-B, D-Asp-D-Nle-Arg-vasopressin (Arg9-vasopressin) and oxytocin. In conclusion, UVB radiation stimulates systemic HPA axis activity leading to increased concentrations of plasma levels of immunosuppressive CORT. The phenotypic consequences of increased levels of URO, CRH and β-End peptides remain to be determined.

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The alpha adrenergic receptor agonist oxymetazoline decreases erythema and inflammation in a UV B-induced wound model
M Tian, E Hsieh, I C Andrews-jones, K Manley, K Azarian, C Cheever and D Col* Allergan, Irvine, CA

The alpha adrenergic receptor agonist oxymetazoline (OX) and brimonidine (BR) are currently in clinical development as promising new therapies for the treatment of erythematous rosacea, which is characterized by erythema and chronic inflammation. The alpha agonists reduce erythema through vasocostriction of cutaneous blood vessels, but their effects on inflammation are not well studied. Using a mouse model of UVB-induced skin inflammation, we tested alpha agonist effects on vasodilatation, erythema, tactile hypersensitivity and inflammation. SKH1 hairless mice were exposed on their right sides to UVB at an intensity of 120 mJ/cm2 for 90 sec. Twenty minutes following irradiation, 0.5% OX or 1% BR were applied topically to 1 cm2 regions of the back and the dorsal surface of the ears. The following assessments were made: 1) Erythema on the back using a chromometer; 2) Tactile hypersensitivity to light stroking of the back with a paint brush; 3) Ear vasculature area using image analysis; and 4) Inflammation by histology. UVB exposure resulted in a 28% increase in ear vasculature area and an 84% compared to sham. Mice were also exposed to UVB radiation; OX inhibited the vascularity at 2 hrs (p=0.013), but not at 4 and 48 hrs post-UVB. Tactile hypersensitivity at 4 hrs post-UVB was reduced by OX (p=0.041), but not BR. UVB exposure also induced vasodilation of the ear vascularity. OX inhibited the vasodilation at 4 hrs (p=0.01) and partially at 48 hrs (p=0.064) post-UVB, but BR inhibited vasodilation only at 4 hrs (p=0.010). The effects of a single topical dose of 0.5% 2-methyl butanol on the ears were also tested. 2-methyl butanol inhibited vascularity at 2 hrs (p<0.01), but not at 4 or 48 hrs.

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Induction of apoptosis by photodynamic therapy using an aluminium-substituted phthalocyanine cyanine dye model
M Tamura, C Matusi and S Georgouss Dermatology, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

Photodynamic therapy (PDT) has recently gained an increasing use in a broad range of applications in oncologic dermatology. PDT requires a chemical compound able to act as a photosensitizer, usually a porphyrin or a phthalocyanine derivative with a pincer-related structure, and the use of a light source of a particular wavelength, suitable for activating the photosensitizer. The effects of PDT are achieved by increasing the oxidative stress and by the induction of apoptosis within the tumoral cells. We aimed to assess the way in which using an aluminium-dil-substituted phthalocyanine (AlSPc) as a photosensitizer leads to the activation the signaling pathways involved in the induction of apoptosis in dysplastic oral keratinocytes. Dysplastic oral keratinocytes (DOK) from normal and inflammatory oral mucosa were treated with AlSPc and examined for apoptosis. The results showed that the AlSPc sensitizer, but not the control sensitizer, led to the activation of the main signaling pathways involved in the induction of apoptosis in dysplastic oral keratinocytes. The signaling pathways examined were JNK, Akt and caspase 3. These results show that the AlSPc sensitizer can be used for the treatment of dysplastic oral keratinocytes.

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Curcumin analog GO-Y030 inhibits UV induced inflammation and carcinogenesis
C Yokozaki, F Yamasaki, W Inoue, H Tsumura, S Ok, K Mizuno, H Shibata, Y Maruho, H Matsunaka, H Okamoto2 Dermatology, Kanazawa Medical University, Kishiwada, Japan, 1 University School of Medicine, Nagasaki, Japan, 2 Clinical Oncology, Akita University, Akita, Japan, 3 Organic Chemistry, Laboratory of Synthesis: Chemistry, Tohoku University, Sendai, Japan and 4 Tokiwa Pharmaceutical Co Ltd, Osaka, Japan

Curcumin, a dietary constituent extracted from turmeric, has a very reasonable tumor suppressive ability including UV radiation, although the mechanism of carcinogenesis inhibition is unknown. The newly synthesized curcumin analog, GO-Y030, showed a 30-fold greater growth suppression of tumors in vitro. We compared the effects of curcumin and GO-Y030 on UV-induced inflammation and carcinogenesis. Since curcumin had a physical sunscreen effect, both agents were applied after UV radiation. HR-1 hairless mice were treated with 1% GO-Y030 or 1% curcumin immediately after 600 mJ/cm2 of UVB radiation. Ear thickness was measured before and 1, 2, 3 days after UVB irradiation. Although there was no difference in the number of tumors between GO-Y030 treated, UVB-injured mice and curcumin-pretreated UVB-injured mice, HR-1 mice treated with GO-Y030 after UVB radiation showed less tumor size (24.5±2.0mm2) than curcumin-treated mice (21.9±2.4mm2) 3 days after UVB exposure. HR-1 mice treated with GO-Y030 30 min before UVB radiation were completely protected from UVB induced tumors. GO-Y030 after UVB radiation showed less ear swelling (25.15±4.34mm) than non-treated mice (29.95±5.25mm) 3 days after UVB exposure. GO-Y030 30 min before UVB radiation and GO-Y030 30 min after UVB radiation showed significant suppression of ear swelling. These results indicated that GO-Y030 inhibits UV-induced inflammation and carcinogenesis more effectively than curcumin.

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Adulthood Cockayne syndrome appears in two cases of UV sensitive syndrome with C229T CSB mutation
I Okazaki,1 D Kudo,2,3 M Iwabuchi,3 H Matsunaka4 and H Okamoto1 Dermatology, Tokyo Medical and Dental University, Tokyo, Japan

Cockayne syndrome (CS) is a rare, autosomal recessive disorder characterized by photosensitivity, growth retardation, immunodeficiency, and neurological abnormalities. It is caused by mutations in CSB. We describe two cases in whom CSB mutations were previously reported in UV sensitive syndromes (UVSS). One case (EIS) had neurological/developmental abnormalities at 39 years of age including hearing loss, ataxia, and dementia. The patient was 33-years-old male and had a history of sun sensitivity, mild freckles and actinic keratoses. The other case (EIIS) had a history of sun sensitivity, mild freckles and actinic keratoses at the age of 39. Both cases revealed intracranial calcification and brain atrophy. Cultured cells from these patients are hypersensitive to UV with normal pre-UV synthesized DNA synthesis and decreased post-UV RNA synthesis. The dysplasia of basal cell nucleation was detected by expression of wild type p53 and genetic analysis showed heterozygous C229T/G343D and homozygous C229T in CSB gene in TMOD and BIS, respectively, resulting in complete absence of CSB protein. Interestingly, C229T/027X in CSB was previously reported in UVSS patients. UVSS 1301 (13-years-old male) and CS3AM (13-year-old boy) without neurological abnormalities or skin tumors. Our findings suggest that UVSS is a late onset CS and patients with UVSS should have neurological abnormalities as in CS and UV-induced skin cancers as in xeroderma pigmentosum in adulthood.

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Repetitive irradiation of infrared-A does not promote ultraviolet induced skin tumor formation in black, yellow, and white K14-SCF mice
S Okazaki, Y Yamasaki, S Oyama and S Kawanuma dermatology, Niigata Medical School, Niigata, Japan

Infrared radiation (IR) is increasingly used for treatment of photoaged skin. Recently, IR-A is reported to confer resistance to UVB-induced apoptosis as well as modify stress signaling. To know the overall affect of IR-A on photoaging, or in two carcinogenesis studies are required. As melanoma and keratinocyte cell proliferation and edema with OX treatment compared to vehicle. Taken together, these data suggest that OX has crosstargeting and anti-inflammatory properties, both of which would be desirable for the treatment of rosacea.

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Study of photodynamic therapy using intraperitoneal injection of 5-ALA for M RSA infected cutaneous ulcer
K Murakami, T Oida, T Morita, K Azawa, K Kuboyama and M Ishii1 Plastic & Reconstructive Surgery, Osaka City University, Graduate School of Medicine, Osaka, Japan, 2 Plastic Surgery, Osaka University, Graduate School of Medicine, Osaka, Japan and 3 Medical Beam Physics Laboratory, Osaka University, Graduate School of Engineering, Osaka, Japan

Photodynamic therapy (PDT) is thought to cause bactericidal action by the cytocidal effect of singlet oxygen and inhibit thrombin synthesis in cellular DNA by a porphyrin derivative. To identify the low possibility of antibiotic-resistant bacteria appearing, and has attracted attention as a new treatment for bacterial infectious diseases. We would like to report the significant promotion of wound healing due to PDT using an intraperitoneal injection of 5-aminolavulinic acid (5-ALA) for M RSA infected cutaneous ulcer which was prepared on the back of a mouse. In this PDT experiment, 5-ALA was used as the photosensitizer and LED with a wavelength 410 nm as the light source. First, M RSA Bacteria count was 0/10 when PDT was performed with an ALA concentration of 1.9 mM, and irradiation output of 50 J/cm2. Next, after a 4 mm full-thickness skin defect injury was created on the back of a mouse with diabetes. An ulcer model was prepared by swaging a silicon ring around the wound as the injury and attaching MBBA. This model was divided into six groups. In ALA group, an ALA concentration of 50 mg/ml and irradiation of 50 J/cm2, Group 2 with an ALA concentration of 50 mg/ml and irradiation of 50 J/cm2, Group 3 with an ALA concentration of 200 mg/ml and irradiation of 50 J/cm2, Group 4 with an ALA concentration of 200 mg/ml and irradiation of 100 J/cm2, Group 5 with an ALA concentration of 50 mg/ml and irradiation of 50 J/cm2 and Group 6 with an ALA concentration of 50 mg/ml and irradiation of 100 J/cm2 was performed every day, and ulcer dimension and bacteria count were compared with the control group. As a result, wound healing in Group 4 with an ALA concentration of 200 mg/ml and irradiation of 50 J/cm2 was more advanced than the control group and bactericidal effect was reduced. Performing PDT using an intraperitoneal injection of 5-aminolavulinic acid (5-ALA) may be useful as a treatment method for M RSA infected cutaneous ulcer.
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Time-dependent changes in the cyclobutane pyrimidine dimers (CPDs) level in mice epidermis by UVB irradiation

E. T. No. 1 M. Tonai, 1 H. Kunimoto, 1 T. Funahashi, 1 and A. Mori, 1 1 Cosmet. and Environmental Dermatology, Nagoya City University, Graduate School of Medical Sciences, Nagoya, Japan and 2 Central Service Unit, Aichi Cancer Research Institute, Nagoya, Japan

It is thought that Cyclobutane pyrimidine dimers (CPDs) formation after UVB irradiation is found at the early time point, likely a few minutes and immediately decreased by DNA repair system. The peak formation is considered at the immediate point after the irradiation. In this study, levels of cyclobutane pyrimidine dimers (CPDs) formation change in skin by UVB irradiation was investigated at the early time point (3 mins) to 24-72 hrs. Two mice strains, B6 and BALB/c mice were used. Their back was shaved and irradiated by narrow-band UVB, broad-band UVB. The skin specimens (n=3) were taken from 3 mins, 6, 9, 12, 18, 24, 48, and 72 hrs after UVB irradiations and stained with an UV-specific antibody. Positive cells in the epidermis are counted by Image. The positive counts were determined. The specimens were also tryptase-induced to separate only the epidermis. DNA was extracted from the separated epidermis and subjected to the quantitative analysis of CPD by ELISA CPDs by UVB irradiation in the nucleus. CPDs level was found immediately after irradiation (3 mins), but higher levels after 3-9 hrs and lasted for 24-44 hours after irradiation. The same trend was observed regardless of strains and differences in mice and the type of ultraviolet to targets gene promoters to enhance UVB skin. The CPDs in the epidermis were observed by other transcription factor, for instance the NF-κB, thereby probably contributing to the development of UV-induced skin inflammation. It is thought that Cyclobutane pyrimidine dimers (CPDs) formation in skin by UVB irradiation is significant and the level of CPDs formation is important to evaluate the UVB-induced skin inflammation.

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The ary hydrocarbon receptor (AhR) suppresses UVB-induced apoptosis in human keratinocytes

I. Haarmann-Stemmann, 1 K. Frauenstein, 1 U. Sydlik, 1 T. Tigges, 1 M. Majura, 1 H. Hanenberg, 2 C. Esser, 1 E. Fritsche, 1 and J. Krutmann 1 1 IUF - Iodized Research Institute for Environmental Medicine, Duesseldorf, Germany

Exposure of keratinocytes (KC) to UV radiation results in the initiation of apoptosis, a protective mechanism that eliminates cells harboring irreparable DNA damage. Hence, a modulation of this process may significantly influence the initiation and progression of UV-induced skin cancer. We have found that the AhR, a ligand-activated and UVB-sensitive transcription factor, serves as an anti-apoptotic function in UVB-irradiated KC. Chemical and siRNA-mediated knockdown of AhR signaling significantly enhanced UVB-induced apoptosis. This effect was due to a loss of expression and function within the downstream target checkpoint kinase-1 (CHK1), two factors critical for cell-cycle control and DNA damage responses. The decrease in E2F1 expression in AhR-knockdown cells was due to an enhanced expression of p27KIP1, accompanied by a reduced phosphorylation of CDK2 and retinoblastoma protein, resulting in an inhibition of E2F1 ubiquitination. Ectopic overexpression of E2F1 in AhR-knockdown KC restored CHK1 expression and diminished the observed sensitization to UVB-induced apoptosis. Accordingly, experimental CH1 recovery alone was also sufficient to suppress UVB-induced apoptosis, which was accompanied by a reduced basal expression of E2F1 and CHK1. Thus, the newly described anti-apoptotic AhR-E2F1-CHK1 axis is also present in vivo. Our results demonstrate for the first time an interaction between AhR, E2F1 and CHK1 and identify this signaling axis as a novel anti-apoptotic pathway in KC, which may represent a putative target for chemoprevention of non-melanoma skin cancer.

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The ary hydrocarbon receptor (AhR) enhances IL-6 expression in UVB-exposed keratinocytes

K. Frauenstein, 1 J. Kemmerling, 1 CS Vogel, 1 J. Krutmann 1 and T. Haarmann-Stemmann 1 1 IUF - Iodized Research Institute for Environmental Medicine, Duesseldorf, Germany

Acute exposure to ultraviolet (UV) radiation induces a variety of inflammatory skin reactions, such as erythema, neutrophil infiltration, vascular remodeling, and secretion of inflammatory mediators. Keratinocytes (KC) are the major epidermal cell-type responsible for the release of pro-inflammatory cytokines, such as IL-8, TNFα, and IL-6. The expression of IL-6 is up-regulated in several inflammatory skin diseases (atopic dermatitis, psoriasis) and it is also involved in the pathogenesis of skin cancer. Here we identify the AhR, a key regulator of drug metabolism, as a novel modulator of IL-6 expression. In contrast to AhR-proficient NCTC144KC, quantitative gene expression analyses revealed a reduced induction (~50%) of IL-6 in AhR-knockdown KC 6 h after exposure to 200 mJ UVB. Accordingly, ELISA-based IL-6 determination showed a significant decrease (~40%) of secreted IL-6 in the supernatant of AhR-knockdown KC 24 h after exposure to 400 mJ UVB. The AhR is activated by tryptophan photoproducts generated in the cytosol of UVB-exposed KC. Upon activation, the AhR shuttles into the nucleus, dimerizes with AhRR, and translocates to the cytosol of UVB-exposed KC. The AhR-knockdown KC were observed by other transcription factor, for instance the NF-κB subunit relA/p65. Interestingly, NF-κB is the major regulator of IL-6 expression in KC. To test if the AhR interacts with NF-κB signaling to induce IL-6, we pre-treated AhR-knockdown KC with BV 12-3955, a potent NF-κB inhibitor, prior UVB-exposure. The UVB-induced IL-6 induction was completely abrogated by BV 12-3955 in all cell-types, demonstrating the dominant role of NF-κB in IL-6 regulation. Thus, it is highly likely that the AhR enhances IL-6 expression by cooperating with NF-κB, thereby probably contributing to the development of UVB-induced skin inflammation.
1263 Fragmented collagen microenvironment alters expression of matrix metalloproteinases and collagen turnover in dermal fibroblasts in photodamaged human skin
T Quan, J Little, H Quan, Z Qin, J Voorhees and GJ Fisher Department of Dermatology, University of Michigan, Ann Arbor, MI Solar ultraviolet (UV) irradiation induces matrix metalloproteinases (MMPs), which fragment collagen fibers that comprise the bulk of the dermal extracellular matrix (ECM). This ECM fragmentation accumulates with chronic sun exposure, leading to permanent structural and functional impairment. We examined the effects of fragmented collagen microenvironment on dermal fibroblast function by culturing dermal fibroblasts in intact or fragmented three dimensional (3D) collagen lattices to model sun-protected and sun-exposed dermis, respectively. Fibroblasts expressed 17 of the 23 known members of the MMP family. Interestingly, gene expression of six MMPs were elevated in fragmented 3D collagen lattice cultures (N=3, p<0.05). These MMPs included MMP1 (4.5-fold), MMP2 (1.4-fold), MMP3 (1.1-fold), MMP11 (3.1-fold), MMP13 (3.6-fold), MMP27 (3.2-fold). Furthermore, type I collagen expression was significantly reduced in fragmented 3D collagen lattices (N=3, p<0.05). To evaluate the physiological validity of the 3D collagen lattice model, we quantified gene expression of the MMPs and MMP inhibitors in sun-protected and sun-exposed human skin. Among the 18 MMPs expressed in human dermis, all six MMPs that were up-regulated in fragmented 3D collagen lattice cultures (N=3, p<0.05). MMP1, MMP2, MMP3 and MMP13 were significantly up-regulated in sun-exposed dermis compared to sun-protected dermis (N=3, p<0.05). These data support the model that constituve elevation of MMP expression and reduced collagen turnover in physically fragmented dermis, which is consistent with the clinical observation of dermal fibrosis in photodamaged skin.

1264 Topical estrogen increases the severity of UV-induced squamous cell carcinoma
TD Hidayat, GL Sobie, TM Rams, AJ Riggenbach, DJ Westfall and TM Ohto-Nezuki
1 University Laboratory Animal Resources, The Ohio State University, Columbus, OH, 2 Pathology, The Ohio State University, Columbus, OH and 3 Molecular Carcinogenesis, Scott Centers for Cancer Research Division - The University of Texas MD Anderson Cancer Center, Smithville, TX
Cutaneous neoplasms, both non-melanoma skin cancer (NMSC) and melanoma, result from UV exposure, account for nearly one half of all cancers in the United States with increasing incidence in women under 40. In addition to causing skin cancer, sunlight has been implicated in the cutaneous aging process. In women, excessive sunlight exposure during youth combined with a decrease in estrogen levels in later life results in pronounced skin wrinkling and loss of elasticity. There is increasing interest in the use of topical creams containing estrogen to prevent or reverse some of the subsequent cutaneous aging processes in younger pre-menopausal women. While exposure to these creams may be beneficial cosmetically, the effect of applying estrogen to sun exposed sites for pro-longed periods of time on skin cancer development has not been extensively studied. The current studies were carried out to examine the effects of topical estrogen (Estrogen®, 17β-estradiol) treatment on inflammation following acute UVB exposure and on skin tumor development in female SKH1 hairless mice following 10 weeks of UVB exposure. We show that topical treatment with estrogen may stimulate UVB-induced inflammatory response. Mice chronically UVB-irradiated and then treated topically with estrogen developed 1.6 fold more tumors, an almost 1 fold greater tumor burden, and tumors of a different morphology. Furthermore, estrogen treatment induced an immune response that is highly dependent on the use of estrogen on previously sun damaged skin for cosmetic purposes may increase skin tumor development and should be used with caution.

1265 Activation of the glucocorticoid-induced leucine zipper in human dendritic cells: Implications for immuno-modulation after extracorporeal photochemotherapy
T Küsterling, RF Tigelaar, J Choi and RL Edelson
1 Department of Dermatology, Yale University School of Medicine, New Haven, CT
Extracorporeal photochemotherapy (ECP) induces antigen-specific immune tolerance in graft-vs-host disease and solid-organ transplantation. We report that ECP acts via two pathways culminating in glucocorticoid up-regulation in human monocytes in response to UVB irradiation. ECP directly induces glucocorticoid expression in an apoptotic cell dose-dependent fashion (p<0.01). Second, ECP acts indirectly through the generation of apoptotic cells to induce glucocorticoid expression in an apoptotic cell dose-dependent manner (p<0.01). MoDCs treated with PUVA, and exposed to apoptotic lymphocytes induced by PUVA, up-regulate GILZ, down-regulate CCR7, and CCR11, become senescent after induced maturation, increase IL-10 production, and decrease production of pro-inflammatory cytokines (IL-12, IFNγ, IL-6, TNFα) and chemokines (IL-8, MCP-1, MIP-1β, RANTES) (p<0.05). Knockdown of GILZ by siRNA reduced GILZ expression in PUVA-irradiated keratinocytes, but not in culture. GILZ inhibition suppressed maturation, chemokine production, and cell-cycle arrest (p<0.05). Knockdown of GILZ by siRNA reduced GILZ expression in PUVA-irradiated keratinocytes, but not in culture. GILZ inhibition suppressed maturation, chemokine production, and cell-cycle arrest (p<0.05). Knockdown of GILZ by siRNA reduced GILZ expression in PUVA-irradiated keratinocytes, but not in culture. GILZ inhibition suppressed maturation, chemokine production, and cell-cycle arrest (p<0.05). Knockdown of GILZ by siRNA reduced GILZ expression in PUVA-irradiated keratinocytes, but not in culture. GILZ inhibition suppressed maturation, chemokine production, and cell-cycle arrest (p<0.05). Knockdown of GILZ by siRNA reduced GILZ expression in PUVA-irradiated keratinocytes, but not in culture. GILZ inhibition suppressed maturation, chemokine production, and cell-cycle arrest (p<0.05).

1266 A three-dimensional epidermis model reconstructed from UVB-irradiated keratinocytes mimics premature aging in human skin
L Lasho, G Weindl and M Scholtz-Karing Institute of Pharmacy (Pharmacology and Toxicology), Free Universität Berlin, Berlin, Germany
The use of 3D skin models to mimic human skin has become an important alternative to in vivo testing in animal models and in vitro testing against non-native substrates. 3D skin models closely resemble human skin in structure and function. They are also more physiological and exhibit increased expression of cytokines and other inflammatory mediators. Additionally, they are used to study the effects of drugs and other substances on skin. In this study, we developed an ex vivo model of 3D human skin using keratinocytes and fibroblasts from human skin. This model was used to study the effects of UVB radiation on human skin and to develop a model that mimics the effects of UVB on human skin. The model was validated using the measurement of UVB-induced MDA levels. The model was shown to be a useful tool for the study of UVB-induced skin damage.

1267 Post-UV delivery of CPD-phosphate mRNA leads to repair of DNA damage in human keratinocytes
M Boros, J Mike, G Emmi, G van der Horst, H Masamatsu, D Weissman, H Horak, G Emmi, K Karaki and PVA Romrend1Department of Dermatology, University of Debrecen, Hungary, 2 Department of Genetics, Erasmus University Medical Center, Rotterdam, Netherlands, 3 Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA
UVB irradiation induces harmful photochemical reactions, including formation of cyclobutane pyrimidine dimers (CPDs) in DNA. Accumulation of unrepairred CPD lesions causes inflammation, premature aging and skin cancer. In response to photoactivated light, photolyase can quickly restore DNA integrity, but due to an evolutionary loss, this enzyme is absent in humans. Naturally, CPDs in human genomic DNA are eliminated by slow nucleotide excision repair (NER). We have recently demonstrated that transplanting in vitro-synthesized mRNA encoding Photoreactive Inertial 3D CPD-phosphate into human keratinocytes leads to rapid repair of DNA damage (induced by UVB at 12 mJ/cm2) posttransfection. In the present study, HaCaT keratinocytes were first exposed to UVB (20 mJ/cm2) then transfected with LipoEctamine-complexed phosphate mRNA. UVB irradiation did not reduce the efficacy of mRNA transfection and a significant amount of phosphate could be measured 1 hour after mRNA delivery by Western blot. At 6 hours following UVB irradiation, we detected a 10-fold reduction CPD lesions in cells subjected to photoactivation compared to those kept in the dark. Thus, confirming that the DNA repair was performed by photoactivation transfection. In comparison, the NER system took 72 hours for the physiological repair. To reduce the amount of CPDs by 90% in UVB-irradiated HaCaT cells, which markedly contrasts the rapid CPD removal facilitated by phosphate mRNA delivery. Our results demonstrate that delivery of in vitro-synthesized mRNA encoding CPD-phosphate is a therapeutic approach for skin cancer prevention that can be applied before or after UVB exposure.

1268 Protein-epitopes formed in sun damaged human skin from lipid peroxidation are mediators of cutaneous photo-oxidative stress
JJ Williams, Y Bermudez, SP Strattan and GT Wondracz1 Department of Dermatology, University of Arizona, Tucson, AZ and 2 College of Pharmacy, University of Arizona, Tucson, AZ
Oxidative stress in human skin exposed to solar ultraviolet light (UV) is a key mechanism underlying cutaneous photocarcinogenesis and arthritis. Malondialdehyde (MDA) is a reactive electrophilic carbonyl species derived from membrane lipid peroxidation. Here we present evidence that MDA formed in cultured skin cells and human skin in response to solar UV-light drives the formation of protein epitopes that are potent photosensitizers. Quantification of MDA in human skin exposed to solar UV-light revealed that control and irradiated cells formed a stratified epithelium as indicated by the expression of differentiation markers (keratin 14, keratin 10, involucrin). However, the epidermis model from UVB-irradiated keratinocytes showed an altered morphology when compared to models reconstructed from normal keratinocytes. The levels of the pro-inflammatory cytokines IL-1α and IL-6 were strongly increased in the models obtained from irradiated cells as determined by ELISA. Furthermore, gene expression analysis by quantitative RT-PCR identified differences in ex vivo exposure of mononuclear cells, compared to sun-exposed UVB-irradiated keratinocytes. These results suggest that reconstructed epidermis from UVB-irradiated keratinocytes may be a useful tool to investigate the physiological properties of prematurely aged skin.

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1269 Mice exposure to repetitive low UV doses differentially affects the immune system compared by elevated dendritic cells, enhancing specific antibody production
EM Celis, ML Paz, AD Friedlich, J Leoni and DH Gonzalez-Mejia. Immunology Department, Pharmacy and Biochemistry School, University of Buenos Aires, Buenos Aires, Argentina
Skin UV exposure induces both a systemic immunosuppression and Vitamin D synthesis, which in turn promotes transcription of immune mediators such as antimicrobial peptides and TLR-2. SKH-1 hairless mice were irradiated with repetitive low UV doses (4 times of 20 mJ/cm²) simulating daily exposure or a single high dose (400 mJ/cm² - simulating harmful exposure) to compare their cellular as well as immune effects, 1 and 8 days post-UV. Both UV doses led to an increase in epidermal thickness (H&E), but only 1×4×20 activated mitochodrial function (3DOM, cells, cell) was markedly decreased in 1×4×00 (flow cytometry); accordingly, epidermal transcription of CXCL-12 was also decreased, but in all mice groups (RT-PCR).

Finally, a tetanus toxoid (TT) challenge was performed for each UV-dose 1 or 8 days after irradiation. In all mice groups, both UV doses induced an increase in VEGF transcription, in dermis and epidermis, but none of them had effects on the transcription of TLR-2 and TLR-4, in both tissues (RT-PCR), or in the 25-OH Vit D serum levels (RIA).

Thus, all tetanus toxoid (TT) challenge was performed for each UV-dose 1 or 8 days after irradiation. Immunizations performed 8 days post-UV did not have effects on specific antibodies titre, in neither group. All differences are expressed vs non-irradiated control mice: *p<0.05, &p<0.001. Simulated daily UV-exposure shares some of the negative effects produced by a harmful high-dose; in contrast it does not induce strong inflammation and it is also unexpectedly capable of increasing the specific humoral immune response.

1271 Extracorporeal photopheresis in the treatment of localized scleroderma
U Just, A Tanew, V Paulitschke, C Jantschitsch and R Knechtler Dermatology, Medical University of Vienna, Vienna, Austria
Extracorporeal photopheresis (ECP) is a treatment for cutaneous T-cell lymphomas, graft-versus-host disease (GVHD), organ transplant rejection, systemic sclerosis, and other immunemediated disorders. Morphoea (localized scleroderma) is a skin disorder with significant morbidity. There is no consistent recommendation proved to be effective or to substantially modify disease progression. Various treatment options are currently used with only partial and often insufficient success. Increasing evidence suggests that ECP can be applied for improving significantly the course of generalized deep scleroderma.

In this prospective single center clinical study we treated 12 patients (age 21-65, male:8, female:4) with severe refractory localized scleroderma in 4×20 sessions every two weeks for a total of six months. Clinical examination as well as high-frequency ultrasound evaluation of skin involvement was performed before initiating the treatment as well as after the 6-month treatment. Histological confirmation was performed in 2 patients with uncontrolled disease progression prior to ECP showed no further progression after starting the ECP treatment. In one patient the symptoms of generalized morphea increased despite treatment. Our data confirm the use of ECP in refractory localized scleroderma is a viable treatment option with negligible side effects. Additional research into the clinical and immunological mechanisms of action of this safe treatment modality should further insight into the mechanisms of action of ECP and the disease itself.

1272 Endoplasmic reticulum-stress induction as a hormetic signal for oxidative stress protection
J Valente, E Lomonte, F Macacaro, N Nicotra and M Freire Jr. Experimental Biology, Monza, Monza and Brianza, Italy
Reinforcing the natural cellular antioxidant defence mechanisms is an attractive way to fight against oxidative stress-mediated accelerated aging. To achieve this, cutaneous cells were exposed to an "hormetic" signal, e.g. an harmless stress stimulus that triggers an antioxidant response without inducing a detrimental unbalance of cellular redox homeostasis. As an organelle warrant of protein quality, endoplasmic reticulum (ER) can detect stress-induced protein damages, and is capable of generating an antioxidant adaptive response called the unfolded protein response (UPR) involved in a specific signalling network. We have designed a specific hormetic compound (HC), which is able to induce a moderate ER stress in human dermal fibroblasts, without inducing any other noticeable cellular stress. Early evidences for the onset of a low-level ER stress were the re-localization of mitochodria in proximity of ER, towards the perinuclear area, which is indicative of a metabolic adaptive response. Activation of UPR specific transducers triggered the transcription of the transcription factor NF-E2-related factor 2 (NRF2) into the nucleus. Nrf2 binds to the antioxidant response element (ARE) to promote the transcription of several antioxidant genes. Upon treatment with the UPR-inducing HC, expression of ARE-activated genes was observed, mostly in fibroblasts and some genes in keratinocytes. Stress resistance of HC-treated fibroblasts was evidenced by challenging them with cytotoxic doses of UV. Cell viability was higher for fibroblasts pre-treated with HC than for non-treated control fibroblasts. Taken together, our results suggest that an hormetic ER-stress induction can be an effective way to reduce accumulation of oxidative stress-mediated damages, and to slow down aging.

1273 miR-24 functionality in skin response to UV irradiation
DN Syed, J Chanchau, RK Lall, MV Adhami, MI Khan and H Mufti Dermatology, University of Wisconsin-Madison, Madison, WI
Solar ultraviolet (UV) radiation is a major environmental skin carcinogen that induces DNA damage and modulates a variety of genes that regulate cell growth, proliferation and apoptosis. The transcriptional regulation of genes is part of the cellular reaction that operates as a defense mechanism against the adverse effects of UV radiation. MicroRNAs (miRNAs) are a group of small non-coding RNAs which regulate gene functions by targeting sequences in their 3′ untranslated regions. Dermis thickness and epidermal thickness has been observed in previous investigations. Little is known about the role of the miRNAs in the regulation of gene expression in response to UV irradiation in human skin. We generated a miRNA profile of UVB-irradiated normal human epidermal keratinocytes (NHKEs) selecting the physiologically relevant UV dose of 40mJ/cm². In preliminary microarray studies, we identified a subset of 44 miRNAs that were differentially expressed (p<0.05) in NHKE, 4 h post UVB exposure. Further validation by qPCR revealed a total of 22 miRNAs that were modulated by UVB. Additional statistical testing showed miR-24 and miR-1292 to be the most significantly modulated miRNAs in UVB-exposed keratinocytes. Next, we established a multi-layered, well differentiated, 3-D human epidermal skin model, comprising of NHKEs and human dermal fibroblasts co-cultured on human skin. Microarray analysis of these constructs post UVB exposure showed a significant decrease in miR-24 expression, confirming previous findings in NHKE monolayer cultures. In situ hybridization studies in tissues derived from patients diagnosed with squamous and basal cell carcinoma demonstrated downregulation of miR-24 in cancer tissues as compared to normal skin. Our data suggest a role of miR-24 in the regulation of UVB induced responses, and a possible correlation between miR-24 expression levels and the occurrence of skin cancer. Further studies are warranted to determine if these results in loss of inhibitory control of proliferative pathways implicated in the pathogenesis of human skin cancer.

1274 Tropical highland blackberry juice protected against UVB-mediated damage in normal human epidermal keratinocytes and in a reconstituted skin equivalent
A Calvo-Castro, A Delgado, J Chanchau, E Vilato, A Perez, M Rojas and H Mufti. 1 Lab. de Ingeniería de tejidos, Centro de investigación in vitro. Instituto Tecnológico de Costa Rica, Cartago, Costa Rica, 2 Dermatology, Universidad of Wisconsin, Madison, WI and 3 Centro Nacional de Ciencia y Tecnología de Alimentos, Universidad de Costa Rica, San José, Costa Rica
Ultraviolet (UV) radiation from the sun, particularly its UVB (290-320 nm) spectrum, is the primary environmental stimulus leading to skin carcinogenesis. Several botanical species with antioxidant properties have shown photoprotective effects against UVB damage. Costa Rica’s tropical highland blackberry (Rubus adenocaulus) contains important levels of phenolic compounds, mainly ellagic acids and anthocyanins, with strong antioxidant properties. In this study, we examined the photoprotective effect of such blackberry juice (BBJ) on UVB-mediated responses in subnormal normal human epidermal keratinocytes (NHKE) and in a three dimensional model (3D) of normal human reconstituted skin. Pre-treatment (2 h) and post-treatment (24 h) of cultured cells with BBJ reduced UVB (25 mJ/cm²)-mediated (i) cyclohexane pyrimidine dimers (CPD) formation, (ii) p53 phosphorylation at Ser15 and (iii) formation of 8-hydroxy-2-deoxyguanosine (8-OHdG), with a strong protective effect in the 3D model. Furthermore, pre-treatment of NHKE with BBJ increased (i) poly(ADP-ribose) polymerase (PARP) cleavage and (ii) activation of caspases 3 and 8. Further, immunohistochemistry studies were performed to establish the significance of these findings in a 3D reconstituted normal skin model, where we confirmed that BBJ decreased CPD formation and increased expression of caspase-14 and other keratinization markers. These data suggest that Costa Rica’s BBJ may possess useful properties against UVB-induced damage to human skin by reducing DNA damage and increasing apoptosis of damaged cells.
1275 Immediate HIF-1α downregulation following UVB irradiation is triggered by NOX1-mediated ROS generation
G Hartlisch,1 M Hoosein,1,2 W Mahbud,1,3 H de Verneuil,1,2 A Taieb,1,2 F Mazurier1,2 and HR Rezani2 1Inserm U1033, BORDEAUX, France and 2University of Bordeaux, BORDEAUX, France

Hypoxia-inducible factor-1 (HIF-1α) is a major transcription factor sensitive to oxygen levels, which responds to stress factors under both hypoxic and normoxic conditions. We have already shown that UBV irradiation has a biphasic effect on HIF-1α protein expression, in which an immediate downregulation of HIF-1α is followed by its upregulation some hours later. Recently, we and others have demonstrated that HIF-1α has an important role in the regulation of keratinocyte responses to UBV irradiation through affecting DNA repair efficiency and apoptotic cell death, suggesting that its spatiotemporal regulation and activation has a substantial influence on the regulation of UV-responsive genes. In this study we investigated the mechanism of HIF-1α downregulation immediately after irradiation. Here, we show that NADPH oxidase-1 (NOX1) activation following UBV irradiation results in increased cytoplasmic ROS level which, in turn, triggers HIF-1α downregulation. Inhibition of NOX1 activation using a NOX1 inhibitor or downregulation of NOX-1 expression using shNOX1 blocks UBV-induced ROS production and consequentially HIF-1α downregulation. Our results have furthermore shown that blocking of poly(ADP-ribose) polymerase (PARP) does not affect UBV-induced HIF-1α downregulation, indicating that its degradation is done independently of PHD activity. However, mutation of two prolines in oxygen-dependent degradation domain (ODD) of HIF-1α blocks HIF-1α degradation, indicating that its degradation is dependent on the presence of two prolines. The immediate HIF-1α downregulation following UBV irradiation was not observed in VHL−/− cells or shVHL-transduced keratinocytes, indicating that the immediate HIF-1α-downregulation is dependent on VHL-mediated ubiquitination. Altogether, these results indicate that HIF-1α downregulation, which is triggered by UBV-induced NOX1 activation-mediated ROS production, is dependent on the presence of two prolines in the ODD domain as well as VHL activity.

1276 Energy metabolism affects keratinocyte responses to UBV irradiation
M Hoosein,1,2 W Mahbud,1,3 F Mazurier1,2 A Taieb,1,2 H de Verneuil,1,2 R Rossignol1,2 and HR Rezani2 1Inserm U1033, BORDEAUX, France and 2University of Bordeaux, BORDEAUX, France and 3E4457, BORDEAUX, France

The common metabolic hallmark of malignant tumour, i.e., the so-called “Warburg effect”, is their propensity to metabolize glucose to lactic acid at a high rate even in the presence of oxygen. Increased glucose uptake usually reflects an increased rate of glycolysis, with conversion of glucose to lactate and decreased conversion of pyruvate to acetyl-CoA, the substrate for mitochondrial oxidative phosphorylation (OXPHOS). Because the dramatic reprogramming of energy metabolism is observed in more than 95% of advanced cancers, understanding the consequences of this energy metabolism alteration in cell biology is of great importance. In this study we wondered whether energy metabolism alteration affects keratinocyte responses to UBV. To this end, we changed energy metabolism from glycolysis to mitochondrial via exercise studies. First experiments focused on ROS generation (flow cytometry) where UBV-treated cells with the dose of 50 mJ/cm2 revealed 12% ROS up-regulation directly after UBV (0 h) and 4 h after UBV reaching reduction of 21% compared to control at the dose of 50 mJ/cm2 (p<0.001). Comparative analysis of catalase activity by colorimetric assay confirmed these observations. On the other hand, investigations regarding the mechanism of action of melatonin showed that it induced the translocation of Nrf2 transcription factor from the cytosol into the nucleus (EUSA) resulting in increased activity of antioxidative enzymes including catalase (CAT), glutathione peroxidase (GPx), heme oxygenase 1 (HO-1), NADPH quinone oxidoreductase 1 (NQO1), superoxide dismutase (SOD) and γ-glutamate cysteine synthetase (γGCS). These results suggest that melatonin not only directly scavenges ROS but also significantly induces the activation of antioxidative enzymes via the Nrf2 pathway uncovering a new mechanism that efficiently regulates the antioxidative response to UBV-induced stress.

1277 WITHDRAWN

1278 Activation of antioxidative enzymes via nuclear erythroid 2-related factor (Nrf2) in UV-exposed human keratinocytes is mediated by melatonin
TW Fischer, K Kleczynski, N Krane and D Zilkens Department of Dermatology, University of Ulm, Ulm, Germany

Melatonin is a ubiquitous molecule with many different functions, including potent antioxidative properties. Due to its lipophilic character, it easily crosses cellular and intracellular membranes reaching particular cell organelles. Therefore, it is able to act protectively right at the site of occurrence of oxidative stress, e.g. under ultraviolet irradiation (UV). Here, we investigated the effects of melatonin on different antioxidative enzymes (NADPH oxidase, heme oxygenase 1, NQO1, glutathione peroxidase) in human keratinocytes. We used keratinocytes from normal donors with and without a history of sun exposure. In both groups, keratinocytes were exposed to sunscreens of different formulations. Our results show that melatonin is able to activate antioxidative enzymes (NADPH oxidase, heme oxygenase 1, NQO1, glutathione peroxidase) under UV exposure. Furthermore, we have shown that melatonin increases the expression of nuclear erythroid 2-related factor (Nrf2) in human keratinocytes. Nrf2 is a crucial transcription factor in the activation of phase-2 and antioxidative enzymes in human keratinocytes. Our findings indicate that melatonin is able to activate antioxidative enzymes in human keratinocytes under UV exposure.

1279 Toll-like receptor-4 promotes ultraviolet radiation induced cutaneous tumor development
E Simans1, TH Nasti,1,2 CA Elmes and N Yusuf Dermatology, University of Alabama at Birmingham, Birmingham, AL

Ultraviolet (UV) irradiation of the skin induces acute inflammation, and is subsequently linked to cancer. Toll-like receptor 4 (TLR4), a component of innate immunity, has an important role in the regulation of keratinocyte responses to UVB irradiation. Changes in TLR4 expression, and its consecutive phase-2 and antioxidative enzymes in human keratinocytes, are sufficiently sensitive to act as an initial screen of sunscreen efficacy. Using the in vitro assay system for testing sunscreens developed in our laboratory we show that melatonin is able to activate antioxidative enzymes (NADPH oxidase, heme oxygenase 1, NQO1, glutathione peroxidase) under UV exposure. Furthermore, we have shown that melatonin increases the expression of nuclear erythroid 2-related factor (Nrf2) in human keratinocytes. Nrf2 is a crucial transcription factor in the activation of phase-2 and antioxidative enzymes in human keratinocytes. Our findings indicate that melatonin is able to activate antioxidative enzymes in human keratinocytes under UV exposure.

1280 An in vitro assay system for testing sunscreens
SA Thirum,1 P Castelli,1 CE Griftiths,1 M Bell,1 M Brown,1 NK Gibb,1 RE Watson1 and MJ Sherratt1 1Dermatology Centre, The University of Manchester, Manchester, United Kingdom and 2Regenerative Medicine, The University of Manchester, Manchester, United Kingdom

In order to identify sunscreen formulations, a system is required that can assess both UVA and UVB absorption properties. We have previously shown that not only does chronic exposure to ultraviolet radiation (UVR) induce profound changes to the ultrastructure of dermal fibrillic microfibrils but that these UV-chromophore-rich assemblies are also susceptible to acute, physically attainable doses of solar simulated radiation (SSR, 290-400 nm) in vitro. Here, we assess if this in vitro system is suitable to test the efficacy of sunscreen formulations using fibrillin microfibril structure as a biomarker. Sus- pensions of microfibrils from photoprotected human skin were exposed to 2.13 kJ/m2 SSR with: i) no protection (SSR); ii) vehicle; iii) UBV-only sunscreen formulation (F1) or; iv) full-spectrum sunscreen formulation (F2). All products were coated onto a quartz plate [1mg/cm2]. Ultrachromatic changes in mean head-to-head distance (periodicity) and fission angle (angle of 1 head repeat) of 60 microfibrils from 3 individuals were measured by atomic force microscopy. Microfibril periodicity was bimodally distributed following SSR exposure (peaks centred at 400nm & 590nm) but unimodally distributed centred at 500nm in unexposed controls. In contrast, this UBV-induced change in periodicity was absent in F1 and F2 populations (F1: unimodal peak, 52nm; F2: unimodal peak, 50nm). No such UBV-protection was apparent when microfibrils were irradiated under vehicle (bimodal peaks centred at 400nm & 590nm). Similarly, the increase in microfibril flexibility which followed SSR exposure (SSR, mean: 134% unexposed control, mean: 141%) was abrogated by exposure under F1 (mean: 144%) and F2 (mean: 141%) conditions. These results, which confirm our previous observations using fibrillin as a biomarker of UBV damage, demonstrate that in vitro assays are sufficiently sensitive to act as an initial screen of sunscreen efficacy.
Toll-like receptor-4 deficiency enhances repair of ultraviolet radiation induced DNA damage in NHK


ABSTRACTS | Photobiology

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Toll-like receptor-4 deficiency enhances repair of ultraviolet radiation induced DNA damage in NHK


UVB radiation. Normal human keratinocytes (NHK) are widely used to study the UV-B response as they have restricted availability and a short lifespan in culture. The HaCaT cell line has been used extensively in vitro for studies of the UV-B responses of human keratinocytes. In our work we compared the stress-induced processes of NHK, the HaCaT cell line and two lines of TLR4 deficient mice to evaluate the role of TLR4 in these processes. NHK were exposed to 200 mJ/cm2 UVB from a broadband Philips TL-12 source. 24h later, biopsies were taken from these subjects and DNA damage was assessed using the comet assay. These findings further suggest that TJ protein expression after UVB exposure is abnormal in PLE and may indicate that an abnormal skin barrier response to sunlight is involved in the aetiology of the condition.

1283

Abnormal epidermal tight junction protein expression in polymorphic light eruption following UVB irradiation

1. Peter C, 2. O’Neill, 3. LE Rhodes and NK Gibbs Dermatological Sciences, University of Manchester, Manchester Academic Health Sciences Centre, Manchester, United Kingdom

UVB dose. Our data indicates an inherent resistance of basal and supra-basal cell layers to UVB-induced CPD and challenges the dogma that reduced CPD in the lower epidermal cell layers is solely due to optical attenuation of UVB radiation by higher epidermal cell layers.

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Constitutive phosphomorphogen protein is involved in the UVB-induced cellular response in human keratinocytes

1. Benjamin D, 2. Polyánka, 3. A Bebes, 4. G Tax, 5. F Nagy, 6. Klemm, 7. Eva A and 8. M Széll 1, 2 Department of Dermatology and Allergology, Faculty of Medicine, University of Szeged, Szeged, Hungary, 2. MTA-SZTE Dermatological Research Group, Szeged, Hungary, 3. Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary, 4. Institute of Medical Genetics, Faculty of Medicine, University of Szeged, Szeged, Hungary

The Constitutive Photomorphogen Protein (COP1) was first described in Arabidopsis thaliana (Arabidopsis) and defined as a central negative regulator of photomorphogenesis: it functions as an E3 ligase and promotes ubiquitine-dependent degradation. Others have previously demonstrated that the human orthologue of COP1 (hCOP1) is overexpressed in cancer cells and represses the p53-dependent tumor suppression. The aim of our study is to determine the role of hCOP1 in the UVB response of human keratinocytes. Therefore we established keratinocyte cell lines where the expression of hCOP1 was silenced. Using Western blot and immunocytochemistry we could demonstrate significantly decreased hCOP1 protein levels in these cell lines. Next, we investigated the photoreactivity of these cell lines and found that one of them had a significantly increased growth rate, suggesting that hCOP1 silencing affected crucial growth regulation pathways in these cells. In the two other hCOP1 silenced cell lines we could demonstrate a decreased level of p53 expression in the control. Upon UVB irradiation p53 expression decreased less in the control and in the hCOP1-silenced cells, but we detected an additive effect of the silencing and the UVB irradiation on the hCOP1 expression of the cells. Parallel with this, p53 level increased in the two other, however this increase was less pronounced in the hCOP1-silenced cells. Our data suggest that hCOP1 is an important component of the cellular UVB response and established cell lines provide a good tool for further investigations to understand its role in UVB induced signaling processes.

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Characterization of UV-B induced cellular processes in a keratinocyte cell line (HaCaT) immortalized with the HPV-E6 oncogene


One of the important functions of human skin cells is to protect the organism from the effects of UV-B radiation. Normal human keratinocytes (NHK) are widely studied as a reliable substitute for primary cultures, however these cells exhibit limitations for some applications. Our aim was to find a valid and easy to use in vitro system in order to study UV-B responses of human keratinocytes. In our work we compared the stress-induced processes of NHK, HaCaT and a newly established keratinocyte cell line, the HPV-KER. We used MTT assay and a real-time cellular analysis system (CELLignence) to detect the effects of UV-B radiation on the viability and proliferation of these cells. We found that the viability and proliferation of HPV-KER cells resembled that of NHK, while HaCaT cells exhibited growth differences. This is possibly due to the fact that the basal p53 expression is low in HPV-KER cells, and can be induced by UV-B similar to NHKs, while HaCaT cells express a constitutively high basal level of p53. In order to understand the different responses in these cells we are currently comparing the UV-B induced expression of known target genes (IL-1, -8, TNF-α, COX2) in these different cell types. Our data suggest that HPV-KER cells, unlike HaCaT cells, show similar characteristics in their UV-B response as NHKs, therefore they may provide a suitable in vitro model for studying keratinocyte UV-B responses.

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UVB-induced p53 mutations are associated with enhanced tumor formation in CHOP/gadd153 knockout mouse skin


The CCAAT Enhancer Binding Proteins (C/EBPs) are leucine zipper transcription factors that regulate cell-type specific gene expression and are involved in cell cycle control, development, and differentiation. One member of this family, C/EBPα, is involved in the control of proliferation and differentiation. CHOP (C/EBP Homologous Protein, also known as C/EBPβ, gadd153 or d13) is a key regulator of the p53 pathway. Levels of p53, its negative regulator MDM2, and downstream target p21, were evaluated immunohistochemically in tumor sections. Upregulation of p53 was seen in tumors from both CHOP KO and WT mice, but in CHOP KO tumors the majority of p53 was mutated (pAb240). Concurrently p21 and MDM2 levels were reduced in the CHOP KO tumors, indicating a defect in cell-cycle and DNA damage checkpoint control. To study the short-term effects of UVB that might contribute to this phenotype, mice were exposed to UVB and the skin harvested at different times between 0.5 and 72 hours. A decrease in apoptosis (as measured by H&E, TUNEL and Caspase-3 cleavage) was observed in both CHOP KO and WT mice, but in the CHOP KO, apoptosis was increased in the UVB-exposed skin. This is possibly due to the fact that baseline p53 expression is significantly lower in CHOP KO skin compared to WT. In the UVB-exposed skin, CHOP KO skin had an intermediate response. Since p53 activation and mutation by UVB are a major cause of skin cancer, we focused on involvement of the p53 pathway. Levels of p53, its negative regulator MDM2, and downstream target p21, were evaluated immunohistochemically in tumor sections. Upregulation of p53 was seen in tumors from both CHOP KO and WT mice, but in CHOP KO tumors the majority of p53 was mutated (pAb240). Concurrently p21 and MDM2 levels were reduced in the CHOP KO tumors, indicating a defect in cell-cycle and DNA damage checkpoint control. To study the short-term effects of UVB that might contribute to this phenotype, mice were exposed to UVB and the skin harvested at different times between 0.5 and 72 hours. A decrease in apoptosis (as measured by H&E, TUNEL and Caspase-3 cleavage) was observed in both CHOP KO and WT mice, but in the CHOP KO, apoptosis was increased in the UVB-exposed skin.
The effect of repair mechanisms on risk of DNA damage during its in vivo two-photon skin imaging

D Halauszka, K Lottoczki, A Barwolgyi, G Nyonggres, A Kolomnicz, R Szipoz, S Karpati and N Winkler 1 Department of Dermatology, Dermatovenerology and Venerology, Semmelweis University, Budapest, Hungary and 2 Department of Dermatology, University of Lubeck, Lubeck, Germany

Two-photon excitation fluorescence microscopy is a novel system for monitoring the morphology and physiological changes in the skin. The near infrared laser beam (750-1100 nm) allows imaging of deeper layers of the skin down to 500-1000 µm. The epidermis and dermis contain numerous endogenous chromophores, such as NADH, melatonin, collagen, elastin, that can be efficiently excited in the near infrared spectral range by two-photon absorption. Due to the different localization, excitation, and emission wavelengths of these chromophores, they are highly distinguishable. In the risk of thermal damage, the major damage mechanism during imaging is associated with the formation of cytotoxic peroxynitrite dimers (CPD) due to multi-photon excitation of chromophores. In our work, we investigated the in vivo safety risk of different laser sources on murine ear skin in the form of time- and angle-dependent in vivo fluorescence microscopy. The samples were exposed by various types of laser beam irradiation, then DNA damage was evaluated using fluorescent antibody against cytotoxic peroxynitrite dimers. The imaging system and the laser setup focused spot size, wavelength and time delay of the laser beam. We also developed our in vivo two-photon microscopy DNA damage, which paves the road for future diagnostic applications of in vivo two-photon microscopy.

Tanning beds use and skin cancer biomarkers—a pilot study

S Kulnik, W Camp, J Hermus, W Cartell, AB Cantor, M Atthar and CA Elmes 1 Department of Dermatology, UAB Birmingham, AL and 2 Department of Dermatology, University of Alabama at Birmingham, Birmingham, AL

University, Budapest, Hungary, 2 Institute for Solid State Physics and Optics of Wigner RCP, Budapest, Hungary and 3 R&D Ultratlas Lasers KB., Budapest, Hungary

Ultraviolet radiation (UVR) induces oxidative stress in human skin and is significantly counteracted by melatonin via the melanotrophic antioxidative system of the skin. Besides oxidative stress, heat shock protein 70 (Hsp70) is another prominent stress protein induced by UV and is highly expressed in human keratinocytes. Here, we evaluated the potential protective effect of melatonin regarding UVR-mediated modulation of Hsp70 in human normal keratinocytes and ex vivo full-thickness skin.

The UVR-induced up-regulation of Hsp70 is modulated by melatonin in human cultured keratinocytes and ex vivo full thickness skin

S Kulnik, S Zwicker, S Tokay, W Williams, M Kapkeiwnicz, R Wolff and TW Fischer 1 Department of Dermatology, University of Lubeck, Lubeck, Germany and 2 Department of Dermatology, Ludwig Maximilian University, Munich, Germany

1291

Accelerated photocuring during photochemistry in animals deficient for MnSOD in their epidermis

A Barwolgyi, K Gormicz, D Halauszka, G Nyonggres, M Wlaszcz, K Scharfetter-Kochanek, S Karpal and D Winkler 1 Department of Dermatology, Venerology and Skin Oncology, Semmelweis University, Budapest, Hungary and 2 Department of Dermatology, University of Ulm, Ulm, Germany

Manganese superoxide dismutase is a mitochondrial enzyme which is vital in elimination of reactive oxygen species. Therefore, the complete lack of this enzyme is lethal in animal models. However, mice with partial or complete deficiency in particular tissues are viable, yet the loss of enzyme function leads to premature senescence and severely altered function of the particular organ. In our experiments, we investigated the effects of extensive PUNA therapy on 23 epidermaly homozygous (+/-) MnSOD knockout mice. 18 hairless mice with normal enzyme activity were used as a control. We aimed to observe differences in photocuring of the two groups as a result of increased oxidative stress. Photochemistry with clinically relevant doses were given to the animals after UVR exposure. The samples were exposed by various types of lasers beam irradiation. The samples were exposed by various types of lasers beam irradiation, then DNA damage was evaluated using fluorescent antibody against cytotoxic peroxynitrite dimers. The imaging system and the laser setup focused spot size, wavelength and time delay of the laser beam. We also developed our in vivo two-photon microscopy DNA damage, which paves the road for future diagnostic applications of in vivo two-photon microscopy.

Alomonal responses to UV-induced DNA damage in Merkel cell carcinoma

S Hsu, K Ona and M Okamoto 1 Department of Dermatology, University of California, San Francisco, San Francisco, CA and 2 Dermatology Research Unit, VA Medical Center, San Francisco, CA

Merkel cell carcinoma (MCC) is a rare skin cancer frequently associated with the Merkel cell polyomavirus (MCCV). The mechanisms by which the virus leads to cancer are unclear, but the photodistributed nature of the cancer suggests that responses to ultraviolet (UV) radiation are important. We investigated two important responses to UV-induced DNA damage—nucleotide excision repair and cellular response to UV damage. MCCs are more sensitive to UV radiation than normal keratinocytes. Comparison of these findings with normal keratinocytes and UV-sensitive keratinocytes indicated that MCC is more sensitive to the effects of UV radiation.

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The effect of repair mechanisms on risk of DNA damage during its in vivo two-photon skin imaging

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**Functional protein pathway activation mapping of normal skin exposed to solar simulated light**

**SC Emptage,1 C Curiel-Lewandrowski,1 J Othman,2 A De La Guardia,1 R Davis,1 and DF Curiel,1**

1Dermatology Research Unit, VA Medical Center, San Francisco, CA; 2 School of Medicine, University of California, San Francisco, CA.

To understand the chronic effects of solar simulated light (SSL) on human skin, we utilized the three-dimensional skin equivalent EpiDerm®FT™ (SkinMedica, Carlsbad, CA). The acute UVB-induced inflammatory response in skin is characterized by erythema, TNF, IL-1, and a MMP-1/TIMP-1 ratio that does not differ from those observed in non-radiated tissues. Taken together, these data demonstrate that extracellular matrix components are equally induced by ECE and RA in EpiDerm™FT™ tissues. Induction of these ECM components may help to prevent damage resulting from photaging.

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**In vivo siRNA targeting of CD28 enhances expression of nucleotide excision repair genes and diminishes DNA damage, hyperplasia, and inflammation induced by UV radiation**

TP Singh and PV Nair.

Dermatology Medical University of Graz, Graz, Austria.

CD28 siRNA targeted CD28 mRNA expression was investigated in vivo in athymic nude mice exposed to solar simulated light. CD28 siRNA treatment resulted in decreased CD28 mRNA expression, reduced CD28 protein expression, and reduced UVB-induced DNA damage and inflammation.

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**TNFs blockade in vivo abolishes UVB-induced recruitment of neutrophils and macrophages to skin, expression of MMPs and MPO, but not MMP13 or collagen fragmentation**

VAMC, Philadelphia, PA and 2 Philadelphia VAMC, Philadelphia, PA.

The acute UVB-induced inflammatory response in skin is characterized by erythema, TNFα, IL-1, COX-2, and a MMP-1/TIMP-1 ratio that does not differ from those observed in non-radiated tissues. Taken together, these data demonstrate that extracellular matrix components are equally induced by ECE and RA in EpiDerm™FT™ tissues. Induction of these ECM components may help to prevent damage resulting from photaging.

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**Protection and repair of skin damage from environmental aggressors**

S Chen, J Ilkovic-Baldis, J DiMaria, T Jacob and U Santhanam.

In vivo protection against photoageing and subsequent skin carcinogenesis.

The acute UVB-induced inflammatory response in skin is characterized by erythema, TNFα, IL-1, COX-2, and a MMP-1/TIMP-1 ratio that does not differ from those observed in non-radiated tissues. Taken together, these data demonstrate that extracellular matrix components are equally induced by ECE and RA in EpiDerm™FT™ tissues. Induction of these ECM components may help to prevent damage resulting from photaging.
Reduction of UVA-induced oxidative stress via regulation of catalase - a novel photoprotective mechanism of α-melanocyte-stimulating hormone in cutaneous biology

M Böhm, A Stegemann, M Mastrofrancesco, M Picardo, A Abdel-Malek and TA Luger

Department of Dermatology, University of Münster, Münster, Germany; 2 San Gallicano Dermatological Institute, Rome, Italy and 3 Department of Dermatology, University of Cincinnati, Cincinnati, OH

Ultraviolet light A (UVA) is a key pathogenetic factor in cutaneous photoaging. We hypothesized that α-melanocyte-stimulating hormone (α-MSH), which previously was shown to reduce UVB-induced DNA damage, may exhibit anti-oxidative effects in human dermal fibroblasts (HDFs) exposed to UVA. We also speculated that photoaging would be increased in red haired pale skin individuals carrying loss of function mutations of the melanocortin-1 receptor (MC1R). HDFs pretreated with α-MSH exhibited significantly reduced intracellular amounts of ROS after UVA exposure. The type of the detected oxidative stress in response to UVA treatment was mainly H2O2 within the cytoplasm as determined by organelle-specific fluoroprobes, incubation with cell-permeable superoxide dismutase, and exogenous catalase. Importantly, a functional MC1R was essential for the suppressive effect of α-MSH on UVA-induced oxidative stress in HDFs. Agouti signaling protein, a natural MC1R antagonist, blocked the protective effect of α-MSH. Accordingly, HDFs carrying loss of function mutations of MC1R (R151C or R160W) displayed increased basal levels of intracellular H2O2 and failed to respond to α-MSH. Importantly, the effect of α-MSH on UVA-induced oxidative stress was paralleled with reduced expression of both MMP1 and 3, key enzymes of dermal photoaging. To finally identify the molecular mechanism by which α-MSH exerts its UVA-protective effect, we performed gene knock-down of catalase. siRNA of catalase completely abrogated the suppressive effect of α-MSH on UVA-mediated accumulation of hydrogen peroxide in HDF. In support of this, α-MSH increased enzyme activity but not mRNA and protein expression of catalase in a time- and dose-dependent manner. In summary, these findings add a novel twist to our current understanding how the cutaneous UVA response is regulated by the α-MSH/MC1R system.