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# 617 The impact of the spectral composition of long-wavelength ultraviolet A1 and visible light on cutaneous biologic effects

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**Antioxidants suppress pro-inflammatory markers induced by ultraviolet radiation and rural pollution in normal human keratinocytes**

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Exposure to ultraviolet radiation (UVR) and airborne pollution, particularly in the form of particulate matter sized 2.5 µm ($PM_{2.5}$), is associated with signs of skin aging and inflammation. Damage from UVR and $PM_{2.5}$ is believed to be the result of oxidative stress, therefore, we examined the effects of a novel composition containing three antioxidants in an *in vitro* model system in order to better understand their potential. We focused in particular on rural sources of $PM_{2.5}$ in order to obtain information related to this source of $PM_{2.5}$. A combination of resveratrol, niacinamide and GHK peptide (AOx mix) reduced UVR-induced oxidative stress in normal human epidermal keratinocytes and cells exposed to rural $PM_{2.5}$ alone and in combination with UVR. The AOx mix increased expression of NRF2 significantly in the presence of $PM_{2.5}$ and UVR, indicating a protective response. RNAseq analysis demonstrated that exposure to ssUV and rural PM downregulates various NRF2-inducible genes and that the antioxidant mix increased transcription of genes that participate in protective antioxidant functions, such as HMOX1, PRDX1, and TXNRD1. The AOx mix was also effective at preventing increases in CYP1A1 and IL-6 after exposure of cells to a combination of UVR and $PM_{2.5}$. These results indicate that a novel topical antioxidant preparation may reduce cellular damage that leads to extrinsic skin aging, not just due to UVR, but also exposure to airborne pollution.

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**Ultraviolet A mediates the keratinocytes supranuclear melanin cap formation via opsin 3**

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Background: the human keratinocytes supranuclear melanin cap acts as a microparasol to protect the nucleus from ultraviolet (UV) induced DNA damage. UV induced cytoplasmic dynein expression mediates supranuclear melanin cap formation. However, the molecular mechanism of keratinocytes responding to UV irradiation and mediating melanin cap formation remains unclear. Opsins(OPNs) belong to the G protein-coupled receptors superfamily and are photosensitive receptor proteins mediating phototransduction by translation of absorbed photons to cellular responses. OPN3 is a member of the opsin family and is widely expressed in mammalian tissues. Its absorption spectrum and function remain unclear. Objective: To investigate whether OPN3 can mediate UVA to induce keratinocytes supranuclear melanin cap formation. Methods: We detected the expression of OPN3 in human primary keratinocyte and HaCaT by real-time fluorescence quantitative PCR and Western blot, and observed the localization of OPN3 by confocal microscopy. silencing OPN3 with small interfering RNA technology and lentivirus transfection technology, ultraviolet irradiation After, the melanin cap formation was observed by Fontana-Masson silver method protocol. And the mechanism of related signal pathway is studied by Western blot. Results: OPN3 is the important light sensor in keratinocytes responsible for UVA mediated supranuclear melanin cap formation. OPN3-mediated melanin cap formation required $Ca^{2+}$-dependent Gai protein-coupled receptor and cyclic adenosine monophosphate (CAMP) signal transduction, thus contributing to the UVA-induced AKT phosphorylation to upregulated cytoplasmic dynein expression, and providing evidence of OPN3 function in mammalian phototransduction. Conclusion: our study provides insights into the molecular mechanisms by which human keratinocytes respond to UVA radiation supranuclear melanin cap Formation and may further reveal the physiological role of skin response to light.

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**The impact of the spectral composition of long-wavelength ultraviolet A1 and visible light on cutaneous biologic effects**

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Background & Aim: Recent studies have demonstrated visible light and long-wavelength UVA1 (VL+UVA1, 370-700 nm) to cause erythema in light skin and synergistically increased pigmentation in dark skin subjects.[1, 2] Spectral compositions of VL+UVA1 may further impact these biologic effects. Yet, no phototesting guidelines exist, thus hindering the development of reliable sunscreens protective against this part of sunlight. The objective of this study was to optimize the spectral output of VL+UVA1 as a step to standardize the assessment of protection from VL+UVA1. Methods: Four subjects with Fitzpatrick skin phototype (SPT) I-III were enrolled in this prospective pilot study. Two VL+UVA1 light sources were used: one with 2% UVA1 and another with 4% UVA1. to match more closely that measured in sunlight. Subjects were irradiated with each light source at 320 J/cm$^2$. Clinical scoring, diffuse reflectance spectroscopy (DRS), and colorimetry were performed immediately, 24 hours, 7 days, and 14 days after irradiation. Results: In all subjects, irradiation with VL+ 4% UVA1 resulted in a stronger cutaneous response than that with VL+ 2% UVA1, showing an average 4-fold and 3-fold increase in immediate erythema and delayed pigmentation, respectively. These results were supported by colorimetry measured Δ a*, Δ ITA, and DRS measured relative dyschromia. Conclusion: These preliminary results indicate that the spectral composition of VL+UVA1 impact cutaneous responses and an output resembling sunlight should be strongly considered when standardizing sunscreen phototesting guidelines. This will enable a realistic and standardized design for the evaluation of sunscreen photoprotection within this spectrum.

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**Macrophage depletion preserves dermal collagen in UVB exposed mice**

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We previously reported that anti-TNFα antibodies (etanercept) block UVB-induced recruitment of neutrophils and macrophages (MΦ) into the dermis in mice. Paradoxically, etanercept accelerates loss of dermal collagen. To examine the role of MΦ in dermal collagen alteration during UVB exposure, we depleted MΦ in mice. C57BL/6J mice were treated with (0.15 ml) of Clophosome-A-Clodronate Liposomes (CCL) i.p. twice, 5 and 3 days before UVB exposure. We had 4 groups- sham, sham+CCL, UVB- and UVB+CCL-treated mice. Mice were UVB-irradiated (100 mJ/cm$^2$/d for 5d) and sacrificed 3h after the last exposure. Skin sections were stained with picrosirius red for collagen fibers. Under circular polarized light, picrosirius red differentiates collagen fibers as red (mature) versus green (thin). In UVB-irradiated mice, red fibers were decreased compared with non-irradiated controls ($p<0.01$). UVB+CCL mice showed more red fibers relative to UVB-treated mice ($p<0.001$). UVB increased collagen fragmentation compared to controls. CCL treatment inhibited UVB-induced collagen fragmentation compared to UVB-treated mice. Procollagen, decorin, and TGF-β protein levels increased in UVB+CCL mice ($p<0.001$) compared to UVB-treated mice. MMP-13 was significantly decreased in UVB+CCL mice compared to UVB-treated mice ($p<0.001$). We further investigated which subtypes of MΦ, M1 or M2, play an important role in maintaining collagen. Imaging mass cytometry showed that MΦ trended to increase after UVB irradiation, as compared to controls and UVB+CCL-treated mice. Overall, we identified CD80+ and iNOS+ M1Φ. Overlapping of these markers suggest M1Φ are one population of cells. M2 MΦ were either CD206+ or ARG-1+, suggesting the presence of two distinct population of M2 MΦ . CD206+ M2 macs were increased with UVB relative to sham ($p<0.05$) and were decreased in UVB+CCL compared to UVB-treated mice ($p<0.05$). In conclusion MΦ depletion maintains mature collagen, with increased levels of procollagen, decorin, TGF-β, and decreased MMP13, suggesting MΦ exert a pro-inflammatory effect in UVB-irradiated skin.

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**CP31398, which reverses UV-induced p53 mutations, does not undo ultraviolet radiation-induced immune suppression**

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Chronic, excessive exposure to solar ultraviolet radiation is the major causative agent for cutaneous squamous cell carcinomas. Mutations in p53 are essential for these cancers to develop. UV radiation is also immunosuppressive and is an additional requirement for UV-induced SCCs to develop. Mutations in p53 can be reversed with the compound CP31398, which results in fewer SCCs in mice exposed to a UV radiation skin carcinogenesis protocol. It is unknown whether p53 mutations contribute to photoimmunosuppression. The purpose of this study was to determine if the UV immunosuppressive effects could be reversed by pretreatment with CP31398. A local UVB regimen consisting of UVB radiation (200 mJ/cm$^2$) for 4 days followed by sensitization with the hapten 2, 4, dinitrofluorobenzene (DNFB) was employed for photoimmunosuppression. To determine the role of CP-31398, we treated the shaved dorsal skin of C57BL/6 mice with CP-31398 (1.25 mg/mouse) or vehicle cream, 30 min before each UVB treatment. Treatment with CP-31398 did not abrogate the immunosuppressive effect of UV. These findings suggest that although p53 mutations are responsible for UV-induced SCCs, they are not necessary for UV-induced immunosuppression.

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**Inflammasome activation in human keratinocytes and mouse epidermis by ultraviolet radiation**

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Ultraviolet B radiation (UVB) is a ubiquitous environmental toxin that causes extensive skin damage resulting in inflammation, photoaging and cancer. UVB is known to induce inflammasome activation and a sterile inflammation associated with cytokine release (e.g. IL-1β, IL-18) and immune cell infiltration. We developed circularly permutated luciferase reporters for caspase-1 activation to assess inflammasome activation by UVB *in vitro* and *in vivo*. HaCaT keratinocytes transduced with caspase-1 reporters encoding either the caspase-7 or Gasdermin D cleavage sequence became active after exposure to UVB, with maximum caspase-1 activation 9 hours post-UVB with 15 mJ/cm$^2$. UVB-induced caspase-1 activation was inhibited by the pan-caspase inhibitor zVAD ($p=0.0002$) and the multi-kinase inhibitor Dabrafenib ($p=0.002$), but not by the pyroptotic pore blocker $LaCl_3$, reactive species scavenger N-acetylcysteine, or intracellular calcium chelator BAPTA-AM. Transfection of Poly I:C induced caspase-1 activation (>80-fold) and resulted in morphological cell death, consistent with the ability of toll-like receptor activation to trigger inflammasome activation and pyroptosis. To evaluate if UVB can induce inflammasome activation *in vivo*, transgenic mice expressing the caspase-7 cleavage sequence reporter were exposed to 50-75 mJ/cm$^2$ UVB and assessed for skin bioluminescence. Caspase-1 activation was observed at 6 hours after UVB exposure, but returned to baseline by 24 hours. This data is surprising given that mouse keratinocytes are reported to not express inflammasome proteins or activate inflammasomes, and the influx of immune cells into the skin peaks 24-72 hours after UVB exposure. Thus, the mechanism of UVB-induced inflammasome activation in mouse skin is unclear. Since inflammation is considered to be a significant driver of tissue damage and cancer formation, understanding how UVB activates skin inflammasomes in both acute and chronic scenarios will provide opportunities to circumvent UVB-induced inflammation and the detrimental photobiology that ensues.