Two in one filter foil: opportunities for protection and therapy

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Preliminary Data and Experimental Setup

It is well known that exposing human skin to solar UVA (320 nm – 400 nm) radiation can induce reactive oxygen species (ROS), cause oxidative damage to DNA like 8-oxoguanosine (8-HdG), DNA double-strand breaks, and lipid and protein oxidation (1). Despite its potential adverse effects, ranging from accelerated photo aging to skin cancer, UV radiation in form of light- and heliotherapy has a continuously increasing spectrum of medical applications (2, 3). Therefore, new UV protective strategies, have to be tested for their efficiency to shield against UV induced damage without reducing its therapeutic potential.

A field that can greatly benefit from improved protection strategies against UVA-induced damage is the Photodynamic Therapy (PDT), a treatment usually prescribed in cases of actinic keratosis (AK), Bowen disease, and certain types of basal cell carcinoma (4). In recent years, Daylight-Mediated Photodynamic Therapy (daylight PDT) has been proposed as an alternative to classical PDT. It substitutes specialized therapeutic devices with a regulated outdoor sun exposure. Daylight PDT proved effective against AK independent of weather conditions and even irradiation reduction by 83% due to cloud-cover resulted in successful treatment as long as a minimal irradiation dose of 3.5-8J/cm2 was achieved (5).

In the current work, we tested HelioVital filter foil that filters up to 60% of solar UVA radiation. The implementation of this foil in a clinical environment can not only increase the sun protection of vulnerable patients but also be used in daylight PDT as it mimics "cloudy" weather conditions.

Experimental Setup

Primary human fibroblasts were irradiated with 6J/cm² or 18 J/cm² UVA with and without HelioVital filter foil. The foil is intended to filter excessive UV-radiation, allowing only small amounts, equivalent to "cloudy" weather conditions, to reach the cells (Fig. 1 a). After the irradiation, cells were collected for MMP, Comet assay, 8-OHdG, and ROS measurement (Fig. 1 b). The irradiation protocols for the different experiments are shown in Fig. 1. 8-OHdG detection was performed by colorimetric ELISA and MMPs were measured vie qPCR.

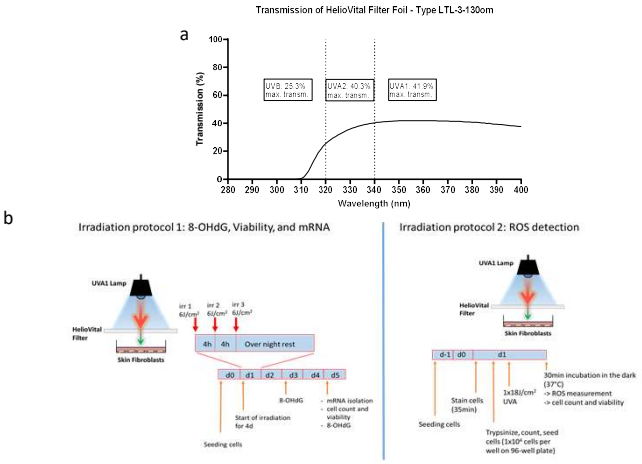

Fig. 1: (a) Transmission of HelioVital filter foil in the range 280-400nm. The filter shows a reduction in transmission for both UVB (280-320 nm) and UVA (320-400 nm) radiation by 60%. (. (b) Graphic representation of the irradiation protocols performed on skin cells. (irr – irradiation).

Results

HelioVital filter foil reduces negative UVA effects on cell proliferation in human fibroblasts

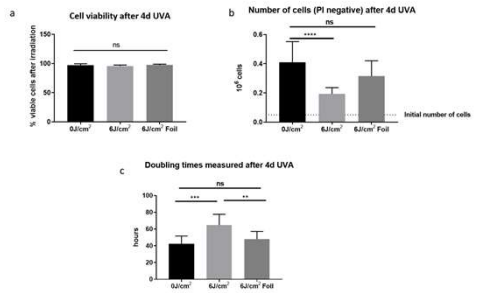

Fig. 2: (a) The repetitive irradiation of primary human fibroblasts with UVA1 has no significant influence on their viability. For this measurement, the number of total cells counted at the end of total treatment (4d UVA1) was compared to the number of live cells detected in the cell suspension and the percent viable cells was derived. (b) The number of primary human fibroblasts is significantly decreased upon UVA1 treatment (4 days of UVA1 irradiation) and this effect can be ameliorated through protection with HelioVital sun protection foil. Since there is no change in the overall viability between treatments (Fig. 2a), differences in the end number of cells are indicative of reduced proliferation rather than apoptosis. (c) In primary human fibroblasts, 4 days of UVA1 irradiation lead to increase in doubling time. The HelioVital sun protection foil can significantly reduce UVA1-induced changes in cell proliferation. (ANOVA with Bonferroni's multiple comparisons test. (ns) P > 0.05; (**) P < 0.005; (***) P < 0.0005; (****) P < 0.0001).

HelioVital filter foil protects against ROS generation during UVA irradiation

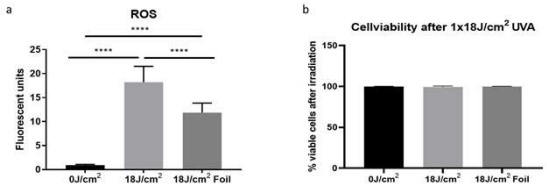

Fig. 3: a) Protection of ROS formation by HelioVital filter foil. In primary human fibroblasts UVA1 treatment induced ROS formation can be reduced when cells are protected with HelioVital filter foil (ANOVA with Bonferroni's multiple comparisons test. (****) P < 0.0001). (b) Despite increased irradiation dose, there was no change in cell viability during the ROS-detection experiment.

Protective effect of HelioVital filter foil against UVA induced DNA damage

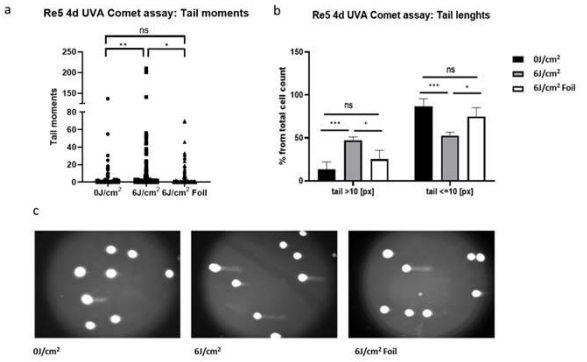

Fig. 4: HelioVital filter significantly reduces the overall tail moment (a) and the formation of large comet tails (tail length >10px) (b) after 4 days of UVA1 irradiation of primary adult human fibroblasts. A minimum of 50 cells were analyzed per condition for each biological replicate (n=3). (ANOVA; p<0.01, Bonferroni's multiple comparisons test. Whiskers represent min. to max. values. (ns) P > 0.05; (*) P < 0.05; (**) P < 0.005; (***) P < 0.0005). (c) Shows three representative images used for the comer-evaluation. (px – pixel)

Protective effect of HelioVital filter foil against the UVA induced DNA damage 8-oxoguanosine (8- OHdG)

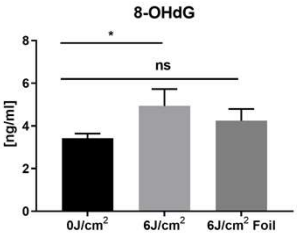

Fig. 5: In primary human fibroblasts, UVA1 irradiation induces formation of 8-OHdG. The amount of DNA damage can be reduced when cells are protected with HelioVital filter foil to levels similar to the untreated control (ANOVA with Bonferroni's multiple comparisons test. (ns) P > 0.05; (*) P < 0.05).

HelioVital filter foil ameliorates UVA effects on the expression of matrix metalloproteinases (MMPs)

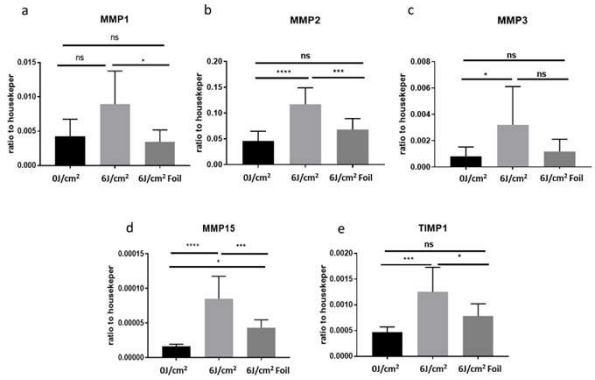

Fig. 6: Primary human skin fibroblasts were irradiated with sub-lethal doses of UVA1 for 410 4 consecutive days with and without protection by HelioVital filter foil. Relative to expression of housekeeper (b-Actin), UVA1 increases the expression of MMP1 (a), MMP2 (b), MMP3 (c), MMP15 (d), and TIMP1 (e). With the exception of MMP1, the UVA-induced expression changes are significant in all other MMPs and TIMP1. The application of HelioVital Filter foil reduces, UVA-dependent, the gene expression significantly in the case of MMP1 (a), MMP2 (b), MMP15 (d), and TIMP1 (d), compared to samples irradiated without protection. MMP3 (c) shows a tendency (albeit not significant) of decreased gene expression in samples protected by HelioVital filter foil compared to UVA-treated unprotected samples. HelioVital-protected MMP3 expression levels are similar to the ones of un-irradiated samples.

Summary and Conclusion

In this work, we investigated the protective effects of HelioVital filter foil against UVA irradiation in skin cells. We could shown that HelioVital sun protection filter foil has protective effects against UVA irradiation induced changes in cell proliferation, MMP expression and against UVA-induced ROS production and DNA damage. These results could pave the way for clinical studies with HelioVital filter foil shielding against the damaging effects of phototherapy and other forms of irradiation therapy, thereby increasing the safety and treatment opportunities of these forms of therapy.

Furthermore, in the context of MMP regulation, it is important to note that, as far as we know from literature, there have been no reports of MMP15 being UVA1-regulated in human fibroblasts. This work is the first describing such UVA-dependent modulation of expression in this type of non-malignant cells.

Literature:
(1) D'Orazio et al (2013). UV radiation and the skin, International Journal of Molecular Sciences, 14(6), 12222-12248.
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(5) Wiegell et al (2011). A randomized, multicentre study of directed daylight exposure times of 1½ vs. 2½ h in daylight-mediated photodynamic therapy with methyl aminolaevulinate in patients with multiple thin actinic keratoses of the face and scalp, British Journal of Dermatology, 164(5), 1083-1090.

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