Photochemistry and Photobiology, 2015, 91: 248–250

Effective Photoprotection of Human Skin against Infrared A Radiation by Topically Applied Antioxidants: Results from a Vehicle Controlled, Double-Blind, Randomized Study†

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Received 14 July 2014, accepted 16 October 2014, DOI: 10.1111/php.12375

ABSTRACT

Infrared A radiation (IRA) from solar sunlight contributes to photoaging of human skin, e.g. by upregulating MMP-1 expression in dermal fibroblasts, indicating the need for photoprotection of human skin against IRA. Up to now, however, there has been no controlled study to show that effective protection of human skin against IRA radiation is possible. Here, we have conducted a randomized, controlled, double-blinded prospective study in 30 healthy volunteers to assess the capacity of an SPF 30 sunscreen *versus* the same sunscreen supplemented with an antioxidant cocktail containing grape seed extract, vitamin E, ubiquinone and vitamin C to protect human skin against IRA radiation-induced MMP-1 upregulation. As expected, exposure to IRA radiation significantly upregulated MMP-1 expression, as compared to unirradiated skin, and this response was significantly reduced, if the SPF30 sunscreen plus the antioxidant cocktail had been applied prior to IRA radiation. In contrast, treatment of human skin with the SPF30 sunscreen alone did not provide significant protection. These results indicate that topically applied antioxidants effectively protect human skin against IRA radiation and that regular sunscreens need to be supplemented with specific antioxidants in order to achieve IRA photoprotection.

INTRODUCTION

It is generally accepted that ultraviolet (UV) rays present in natural sunlight might damage human skin and as a consequence, sunscreen products have been developed to protect human skin against UVB (290–320 nm) and UVA (320–400 nm) radiation. More recently, however, it has been appreciated that radiation in the near infrared range, i.e. IRA (770–1400 nm) also contributes to actinic damage of human skin. This conclusion is based on a steadily growing number of studies providing compelling evidence *in vitro* in human skin cells as well as *in vivo* in mouse and human skin that IRA radiation might cause skin aging (for a recent review see (1)).

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†This paper is part of the Special Issue commemorating the 65th birthday of Dr. Craig A. Elmets.

Thus, photoprotection of human skin should include protection against IRA radiation. Mechanistic studies have revealed that IRA radiation-induced skin damage is initiated by the generation of reactive oxygen species (ROS) in mitochondria of dermal fibroblasts (reviewed in (2,3). Topical antioxidants might thus be useful to protect human skin against IRA radiation-induced skin damage (1). Consistent with this assumption, we previously showed in *in vitro* studies employing primary human skin fibroblasts that selected antioxidants can protect against IRA radiation, e.g. by preventing or reducing IRA radiation-induced expression of matrix metalloproteinase-1 (MMP-1) expression (1,4). We previously also provided proof of principle that topical application of an antioxidant mixture containing vitamin C, vitamin E, ubiquinone and a grape seed extract effectively prevented IRA radiation-induced MMP-1 mRNA expression *in vivo* in human skin (5). Although more and more sunscreens and daily skin care products employing topical antioxidants and claiming IRA protection have been launched worldwide since 2006, a controlled clinical study demonstrating the efficacy of topical antioxidants for IRA protection is lacking. Also, it is currently not known whether conventional sunscreens can provide IRA photoprotection or whether the addition of topical antioxidants is required to achieve this goal.

MATERIALS AND METHODS

In vivo irradiation. This randomized, controlled double-blinded *in vivo* study was carried out in adherence to the Declaration of Helsinki Principles and the International Conference on Harmonization Good Clinical Practice guideline was observed insofar as applicable. The study was approved by the local ethical committee of the Medical Faculty of the Heinrich-Heine-University in Düsseldorf, Germany. The study was approved by the local ethical committee of the Medical Faculty of the Heinrich-Heine-University in Düsseldorf, Germany. After obtaining informed consent, healthy human volunteers ($n = 30$; 11 males, mean age 45.0 years; 19 females, mean age 51.2 years) were treated for 10 consecutive days on two sites of the buttock with 2 mg cm^{-2} of either an SPF30 sunscreen (aqua, propylene glycol dicaprylate/dicaprate, alcohol denat., caprylic/capric triglyceride, ethylhexyl salicylate, octocrylene, butyl methoxydibenzoylmethane, glycerin, methyl glucose sesquistearate, diethylhexyl butamido triazone, cetyl alcohol, tocopheryl acetate, panthenol, cetyl palmitate, hydrogenated coco-glycerides, bis-ethylhexyloxyphenol, methoxyphenyl triazine, *Vitis vinifera* seed extract, tocopherol, ubiquinone, lecithin, ascorbyl tetraisopalmitate, diisopropyl adipate, hectorite, disodium EDTA) including an antioxidant mix (available on the market as Ladival® normale bis empfindliche Haut 30) or the same sunscreen formulation without the antioxidant mixture. The

antioxidant mixture consists of vitamin E, grape seed extract (*Vitis vinifera*), ubiquinone and vitamin C. Both galenic formulations, i.e. the SPF30 sunscreen and the SPF30 sunscreen supplemented with the antioxidant mixture were provided by Dr. Peter Hansen (R&D, Stada, Bad Vilbel, Germany). The two formulations have been allocated by simple randomization for each volunteer in an orange and green plastic bottle by the sponsor. The left buttock was treated on the upper part (site 1) with the product in the green bottle, while the lower site 2 was treated with the product from the orange bottle. Twenty minutes after the last treatment, volunteers were exposed to a single dose of 360 J cm^{-2} IRA from a water-filtered IRA irradiation source (Hydrosun 500; Hydrosun Medizintechnik, Müllheim, Germany) as previously described (5). This device emits wavelengths between 760 and 1440 nm (Figure S1 in (5)) without any contaminating UV radiation as controlled by means of a UVAMETER (Mutzhas, Munich, Germany) and a UV-Dosimeter Type I equipped with a UV6 sensor (Waldmann Medizintechnik, Villingen-Schwenningen, Germany). The IRA output was determined with a Hydrosun HBM1 (Hydrosun Medizintechnik) measuring device and found to be 105 $mWcm^{-2}$ at-a-lamp- to-target distance of 40 cm. For control and sham treatments (site 3 and 4), the volunteers' right buttock skin was either treated with IRA only (site 3, upper buttock) or left unirradiated (site 4, lower buttock). Twenty-four hours post irradiation, 4 mm punch biopsies (n = 4/volunteer) were taken from the sham irradiated (control) skin site, from the irradiated but untreated skin site, from the irradiated, sunscreen pretreated site and from the irradiated site that had been pretreated with the sunscreen formulation supplemented with the antioxidant mixture.

RNA extraction and reverse transciptase-PCR: Gene expression was analyzed according to Marionnet *et al.* (6). In brief, total RNA was extracted from frozen biopsies after disruption in lysis buffer from PeqGold Total RNA Kit (PeqLab, Erlangen, Germany) using a MixerMill MM300 (Retsch, Haan, Germany) three times for 3 min with 30 Hz. 50 ng total RNA were used for cDNA synthesis. PCR reactions were performed in an Opticon 1 (MJ Research, Waltham, MA) with Sybr QPCR Supermix w. Rox (Invitrogen, Karlsruhe, Germany) using specific primer pairs as follows:

18SrRNA 5′-GCCGCTAGAGGTGAAATTCTTG-3′/5′-CATTCTTGGCAAATGCTTTCG-3′ (X03205);
MMP1 5′-CATGAAAGGTGGACCAACAATTT-3′/5′-CCAAGAGAATGGCCGAGTTC-3′ (NM_00 2421.3).

PCR conditions were as follows: activation of hot start taq polymerase 94°C 15 min followed by 45–50 cycles of denaturation, 95°C, 20 s; annealing, 55°C, 20 s; extension, 72°C 30 s. For quantification, the $2^{-\Delta\Delta C_T}$ method was used according to (7).

Statistical analysis. Statistical evaluation was performed with Kruskal–Wallis one way analysis of variance on ranks followed by Tukey's *post hoc* multiple comparison tests with $^*P < 0.05$ compared to sham or as indicated. Data are given as mean ± SE.

RESULTS

In comparison to sham irradiation, exposure to IRA radiation significantly increased MMP-1 mRNA expression in skin areas which had not been pretreated with any of the test products (Fig. 1), thus confirming previous observations (1,5). Similarly, significant MMP-1 mRNA upregulation was also observed in skin areas which had been pretreated for 10 days with the SPF30 sunscreen product, and this IRA response was not significantly different from the one observed in the irradiated, but untreated skin areas. In marked contrast, pretreatment of skin with the same SPF30 sunscreen, which, however, had been supplemented with an antioxidant mixture consisting of grape seed extract, ubiquinone, vitamin E and vitamin C significantly ($P < 0.05$) reduced IRA radiation-induced MMP-1 mRNA expression, as compared with, untreated IRA-irradiated skin (Fig. 1). In fact, in comparison with untreated, unirradiated skin, in these test areas no significant IRA response could be detected.

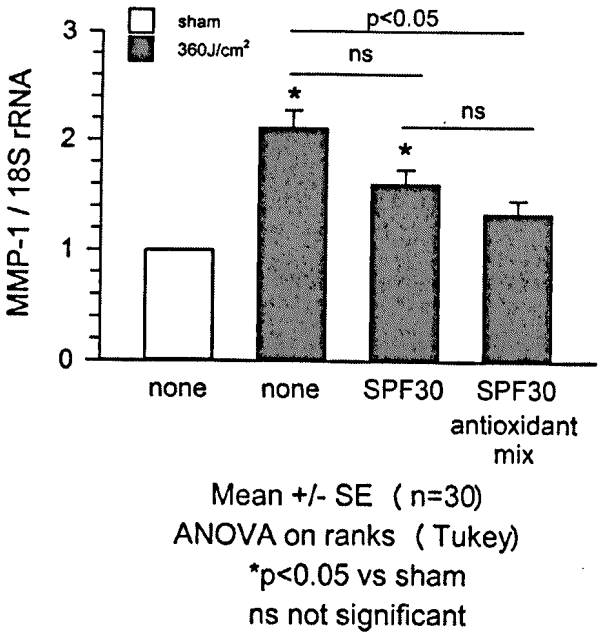

Figure 1. Topical application of an SPF30 sunscreen containing an antioxidant mixture significantly protects human skin (n = 30) against IRA radiation-induced MMP-1 mRNA expression.

DISCUSSION

In this controlled, randomized, double-blinded study we unequivocally show that topical application of an SPF30 sunscreen containing an antioxidant mixture provides significant protection against IRA radiation-induced MMP-1 mRNA expression *in vivo* in human skin. Our results thus corroborate and extent previous data from an uncontrolled, proof-of-principle study employing only nine human volunteers (5) that topical application of appropriate antioxidants might effectively protect human skin against detrimental effects induced by IRA radiation.

In the present study, this conclusion is further supported by the observation that treatment with the identical sunscreen preparation, however, without the antioxidant mixture failed to provide significant protection. As indicated in Fig. 1, there is, however, some protection by the SPF30 product without the antioxidant cocktail as a trend (not reaching significance). We cannot rule out at this stage that other sunscreens, which e.g. contain physical filters capable of reflecting in the IRA range, might provide some protection; the current study strongly indicates that effective protection of human skin against IRA radiation requires that appropriate antioxidants are being applied to human skin.

In this study we have used IRA radiation-induced MMP-1 mRNA expression as a biological endpoint because it is directly related to IRA radiation-induced skin damage in general and skin aging, in particular (1). Despite the invasive nature of our study we strongly believe that this endpoint reflects best IRA radiation-induced skin damage and that the current study strongly supports the conclusion that topical antioxidants are a mainstay of products for photoprotection of human skin. It should be noted, however, that IRA protection claims are not yet regulated and that thus, besides demonstrating efficacy, further attempts are clearly required to standardize IRA protection, e.g. by defining a suitable

protection factor so that IRA protection will become easier to judge for the consumer.

Acknowledgements—This work was supported by the Deutsche Forschungsgemeinschaft (Kr871/5-1 to JK) and an unrestricted grant to IUF provided by STADA AG, Bad Vilbel, Germany. The excellent technical assistance of Heidi Brenden was highly appreciated.

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