Non-Sunscreen Photoprotection: Antioxidants Add Value to a Sunscreen

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The association between ultraviolet radiation (UVR) exposure and both skin cancer and photo-aging is well documented. In addition to the conventional organic-chemical and physical-mineral type sunscreens, other non-sunscreen protective strategies have been developed. These include topically applied botanical extracts and other antioxidants as well as topical DNA repair enzymes. Standard terms of photoprotection such as sun protection factor (SPF) do not accurately reflect the photoprotection benefits of these materials. For example, in spite of minimal SPF, tea extract containing polyphenols such as (−)-epigallocatechin-3-gallate (EGCG) has been shown to protect against UV-induced DNA damage and immune suppression, in part through its ability to reduce oxidative stress and inhibit NF-kB. The addition of botanical antioxidants and vitamins C and E to a broad-spectrum sunscreen may further decrease UV-induced damage compared with sunscreen alone. These agents have been shown to enhance protection against UV-induced epidermal thickening, overexpression of MMP-1and MMP-9, and depletion of $CD1a^+$ Langerhans cells. Non-sunscreen materials such as botanical extracts, antioxidants, and DNA repair enzymes can contribute value when applied topically to human skin *in vivo*.

Journal of Investigative Dermatology Symposium Proceedings (2009) **14,** 56–59; doi:10.1038/jidsymp.2009.14

INTRODUCTION

There is growing interest in the potential for ''non-sunscreen'' agents to add protection against exposure to ultraviolet radiation (UVR). This review summarizes several salient points about this issue, including how non-sunscreen agents are different from sunscreens in their mechanism of action, in the different end points important in measuring efficacy, and in the benefits of their use. In addition, a preliminary study in which antioxidants were added to a broad-spectrum sunscreen with a sun protection factor (SPF) of 25 is reported.

It is important to decrease UV exposure by avoiding excessive time in the sun, wearing protective clothing, and using a sunscreen. It should also be noted that no sunscreen is effective in reducing total UVR exposure under every scenario. This would suggest that sunscreens are to be recommended, but that there are additional topical measures that can be taken to further reduce damage that ordinarily would lead to photo-aging and skin cancer.

It has been suggested that augmenting sunscreens with active natural ingredients can help increase the photoprotective qualities of sunscreens and offer greater protection to patients, but there has been little clinical research to show: (a) exactly what ingredients, (b) at what concentration, (c) combined with which other materials, are beneficial to human skin.

A cascade of reactions occurs on exposure of human skin to sunlight, primarily owing to the ultraviolet wavelengths (UVRs). It is well known that UVR induces an array of damage ''end points'' in human skin, including pyrimidine dimers, oxidative DNA damage, mtDNA damage, release of pro-inflammatory and immunosuppressive cytokines, isomerization of trans-urocanic acid to the immunosuppressive *cis*-urocanic acid, and p53 mutations, in addition to the easily observable erythema on which SPF is based. It is sensible, therefore, that many of the non-traditional sun protection agents be tested against these additional end points for possible protective capabilities. Wavelengths in the UVA portion of the spectrum (320–400 nm) have been shown to induce oxidative stress through reactive oxygen species (ROS), including singlet oxygen, and other non-radical and radical ROS, such as hydrogen peroxide and the superoxide radical. (Grether-Beck *et al.*, 1996; Berneburg *et al.*, 1999; Klotz *et al.*, 2001; Bachelor and Bowden, 2004; Halliday, 2005). This oxidative stress leads to DNA lesions such as 8-hydroxy-2′deoxyguanine (8-OH-dG), which has been proposed as a critical source of mutations, as UVA penetrates

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Abbreviations: 8-OH-dG, 8-hydroxy-2′ deoxyguanine; LC, Langerhans cells; MED, minimal erethema dose; ROS, reactive oxygen species; SS, sunscreens; SS + AOx, sunscreens + antioxidants; UVR, ultraviolet radiation

Received 14 November 2008; accepted 18 December 2008

farther than UVB into the skin, and it may transform stem cells (Agar *et al*., 2004).

In an attempt to block some of the damage caused by sun exposure, antioxidants have been explored as a means to deal with UVR-induced oxidative stress, and UVA radiation in particular. Some topical and systemic antioxidants include resveratrol, vitamin E, vitamin C, (−)-epigallocatechin-3-gallate, and retinyl palmitate (retinoids) (for reviews, see Afaq and Mukhtar, 2006 and Wright *et al*., 2006). Some materials are more accurately referred to as plant extracts (although there is clearly an overlap with the previous category), and they have also been reported to protect the skin against various UVR-induced damage end points. The extracts best supported by experimental evidence include tea extracts (Elmets *et al*., 2001), lutein (Lee *et al*., 2004), tamarind (Kuchel *et al*., 2005), certain flavonoids (Moore *et al*., 2006; Lin *et al*., 2008), fern extract (Caccialanza *et al*., 2007; Siscovick *et al*., 2008), pycnogenol (Sime and Reeve, 2004), and lycopene (Stahl and Sies, 2007). Topical DNA repair enzymes were first introduced over a decade ago as potential therapies for UV-induced damage, specifically for those patients who are genetically deficient in repair enzymes (xeroderma pigmentosum) (Yarosh *et al*., 2001). Since then, the variety of available repair enzymes has increased (to include those targeting oxidative DNA lesions), along with the increased sophistication of delivery systems and the more widespread commercial use of this technology (Yarosh *et al*., 2005; Ke *et al*., 2007). Recently, our group reported on the role of topical DNA repair enzymes in preventing UV-induced immunosuppression (Lucas *et al*, 2008). Topical application of repair enzymes, although effective only after UV exposure, may be an important new weapon in the fight against sunlight-induced skin cancer.

RESULTS AND DISCUSSION

To test the hypothesis that non-erythema end points indicative of UV damage could be prevented better by the addition of antioxidants to a commercial sunscreen, a preliminary study was performed. Five volunteers were enrolled after written informed consent. The mean age was 25, and the range was 18–40 years. Subjects were of Fitzpatrick skin types I-III, primarily II. The mean minimal erythema dose (MED) was 52 mJ cm^{-2}, with a range of 20–60 mJ cm^{-2}. Subjects were treated with two test products: (1) a commercially available skin moisturizer with an SPF of 25 also containing antioxidants (that is, sunscreens plus antioxidants); and (2) the same SPF 25 moisturizer without the antioxidants (that is, sunscreens alone).

Immune suppression has been shown to be a critical aspect of UVR-induced non-melanoma skin carcinogenesis. Depletion of epidermal Langerhans cells (LC), the skin's antigen-presenting cells, has been used as a surrogate for immune suppression, although functional disturbances may exist before the numbers are significantly decreased. In the preliminary study reported here, LCs were quantified to reflect the efficacy of the test materials in protecting against UVR-induced immune suppression. Both sunscreens alone (SS) and sunscreens + antioxidants (SS + AOx) resulted in significant protection against solar-simulated UV-induced reduction in LC numbers in human subjects (Figure 1). The average UV-induced reduction of LC in skin irradiated with two times the MED was 35% compared with non-irradiated sites. In sites pre-treated with SS alone or with SS + AOx, the average reduction of LC was 0 and 4%, respectively. There was no significant difference between the two treatments.

UVR has been shown to activate matrix metalloproteinases (MMPs), and recent studies have determined that MMP1 is the major enzyme implicated in collagen damage and photo-aging of UV-irradiated human skin (Brennan *et al*, 2003). Therefore, this study also asked whether the addition of antioxidants to an SPF 25 sunscreen would improve protection against solar-simulated UVR-induced activation of MMP1. Both sunscreens and sunscreens + antioxidants reduced the expression of MMP1 relative to unprotected UV-irradiated control skin (Figures 2 and 3). SS alone decreased the level of MMP1 per area by 43%, whereas SS + AOx diminished MMP1 production by 60%. The difference in protection between the SS alone and the SS + AOx was significant, and suggests that additional benefit against sun damage can be gained by adding antioxidants to sunscreens. It has been known for some time that UVA is particularly efficient at inducing ROS and degradative dermal enzymes, so it is logical that antioxidants might prevent this end point in particular (Scharffetter *et al*, 1991). In theory, topically applied antioxidants should reduce the damage caused by ROS, impede or lessen tissue damage, and promote repair after UVR. This study shows that it is possible to increase protection from UV radiation by a broad-spectrum SPF 25 sunscreen product through the addition of selected antioxidant botanical ingredients. Further, as the SS + AOx formulation is a commercially available product that has been shown to be stable, safe, and efficacious, this suggests a real developmental advance in sun protection.

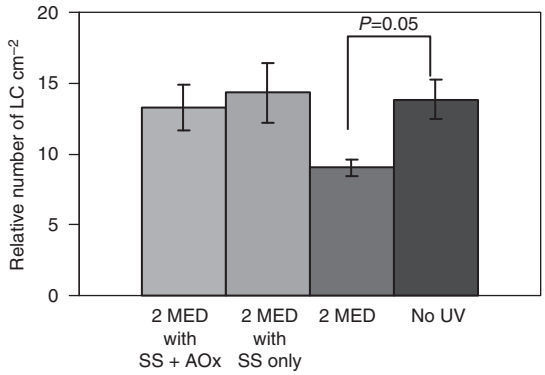

Figure 1. Effect of Sunscreen and Antioxidants on Langerhan's cell number (CD1a+ cells). The skin of human subjects was exposed to 2 MED of ssUVR after pretreatment with the indicated topical agents. Langerhans cells were enumerated as described in Materials and Methods and indicated here as the average per high-powered field ± SD. Both sunscreens alone (SS) or sunscreens + antioxidants (SS + AOx) protected against UV-induced depletion. The difference between the two sunscreen formulations was not significant.

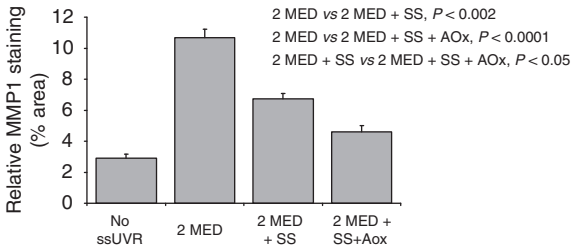

Figure 2. **The effect of ssUVR, sunscreens, and sunscreens plus antioxidants on MMP1 expression in human skin *in vivo*.** The skin of human subjects was treated as indicated: (**a**) no ssUVR, (**b**) 2 MED ssUVR, (**c**) 2 MED plus SS + AOx, and (**d**) 2 MED + SS alone. Biopsies were analyzed by immunohistochemistry using a monoclonal antibody to MMP1 and processed according to the manufacturer's suggestion (R & D Systems Inc., Minneapolis, MN, USA). Measurements were obtained for 8–20 × areas within each tissue section, and the averages calculated. Average values ± SEM are shown after analysis using Image-Pro Plus (Media Cybernetics Inc., San Diego, CA).

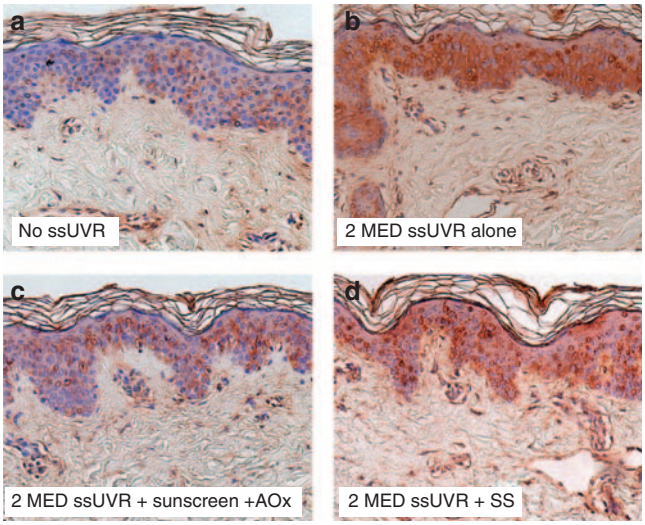

Figure 3. **A representative image of immunohistochemical analysis of MMP1 expression in human skin *in vivo*.** The skin of human subjects was treated as indicated: (**a**) no ssUVR, (**b**) 2 MED ssUVR, (**c**) 2 MED plus SS + AOx, and (**d**) 2 MED + SS alone. Biopsies were analyzed for the presence of MMP1 and processed according to the manufacturer's suggestion (R & D Systems Inc.). Hematoxylin is shown as blue–purple and MMP1 as brown.

MATERIALS AND METHODS

All procedures were approved by the Institutional Review Board of University Hospitals of Cleveland.

Products tested

Two formulations with an SPF of 25 were tested, one with added antioxidants (SS + AOx) and one without (SS alone). The UV filters were benzophenone, avobenzone, and octylmethoxycinnamate. The antioxidants were caffeine, vitamin E, vitamin C (aminopropyl ascorbyl phosphate), echinacea pallida extract, gorgonian extract, and chamomile essential oil. The label SPF was determined by CPT Laboratories (Fairfield, NJ) following FDA guidelines.

UV light source

ssUVR was delivered using a 1,000 Watt xenon arc solar simulator model 6271 (Oriel Instruments, Stratford, CT), with a dichroic mirror and 81017bis filter (WG320 per 1.5 mm), producing a spectrum of 290–400 nm. Irradiance was measured using an IL1700 radiometer (International Light, Newburyport, MA) equipped with a sensor for UVA (SED 033, UVA filter no. 19672) and UVB (SED 240, UVB filter no. 15541) positioned 10 inches from the light source.

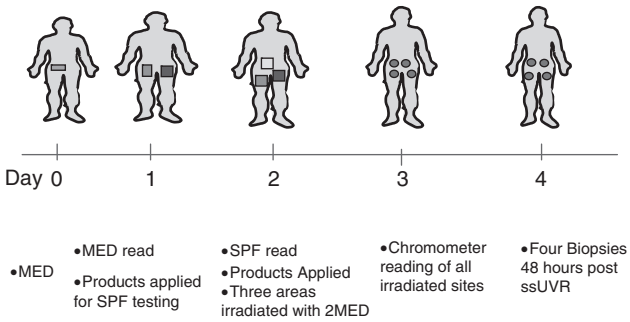

Figure 4. **Diagram illustrating the experimental protocol followed for the clinical aspects of the study.** On day 0, a standard MED determination was initiated and erythema was used to determine the subject's MED 24 hours later. On day 1, products were applied at 2 mg cm^{-2} over two 6 × 8 cm areas. The material was left to dry for 15 minutes, and then the sites were irradiated for determination of product SPF. On day 2 after SPF was determined, products were applied on different sites, and both product and no-treatment control sites were irradiated with 2 MED. Day 3 consisted of chromameter readings of three sites. On day 4, four 6 mm punch biopsies were taken, one from each of the four sites.

Protocol

A schematic summary of the study protocol is provided (Figure 4). Individual baseline MED and SPF determinations were performed (days 0, 1). On day 2, each of the two test products (SS and SS + AOx) was applied on separate areas on the buttock at a dose of 2 mg cm^{-2}. Fifteen minutes later, simulated solar radiation (ssUVR) was delivered on these sites as well as on a third site that was not pre-treated with any product. For the product-treated sites, the dose of UV delivered was calculated as 2 × MED multiplied by the SPF of the product, to account for the UV filtration provided by the sunscreens. Each site measured 2.5 × 2.5 cm. A fourth site was marked but not exposed to UV to serve as control. Two days after irradiation (day 4), skin punch biopsies were obtained from the four skin sites mentioned above.

Tissue analysis

Immunohistochemical staining for CD1a+ cells (LC) and MMP1 was performed on frozen sections from the punch biopsies. Acetone-fixed 4–6 μm cryostat sections were stained using a Vectastain Elite ABC reagent kit that contained blocking serum (Mouse IgG, PK-6102), biotinylated secondary antibody, and Avidin DH/biotinylated HRP (Vector Labs, Burlingame, CA). Samples were incubated with a purified mouse anti-human CD1A monoclonal antibody 1:500 dilution (Immunotech, Cat. No. 1590, Emeryville, CA). Mouse IgG2a kappa, M-7769 was used as isotype control (Sigma-Aldrich Inc., St Louis, MO). For MMP1 detection, samples were incubated in mouse anti-MMP1 monoclonal antibody or mouse IgG1 isotype control overnight (R&D Systems Inc., Minneapolis, MN: diluted 1:250 in 3% normal horse serum in PBS). Peroxidase localization was performed using DAB (Cat. no. SK-4100, Vector Labs, Burlingame, CA), and counterstaining was applied using methyl

green. The number of CD1a+ cells in the epidermis and the percentage of MMP1+ (brown)-stained areas in the epidermis and dermis per high-powered field were calculated using Image-Pro Plus (Media Cybernetics Inc., San Diego, CA). Data were compared between control and UV-irradiated skin, as well as product-treated skin samples using standard *t*-testing. A *P*-value of <0.05 was considered significant.

CONFLICT OF INTEREST

M Matsui is a full-time employee of the Estee Lauder Companies. K Cooper and E Baron have received consulting fees from the Estee Lauder Companies.

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