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Immune Protection Factor of Sunscreens in Humans is Dependent on Protection from UVA and Cannot be Predicted from the Sun Protection Factor

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Sunscreens have been advocated as an important means of preventing skin cancer. UV-induced immunosuppression is important for skin cancer development, yet the effectiveness of sunscreens in protecting the human immune system from UV radiation is unclear. The only currently accepted method of sunscreen rating is the Sun Protection Factor (SPF) based on prevention of erythema. We developed an *in vivo* non-invasive method for evaluating protection of the human immune system from UV radiation based on recall contact sensitivity to nickel, a common allergen. Using this system we showed that broad-spectrum sunscreens provide greater protection to the immune system than sunscreens which protect from UVB only. UVA was found to be immunosuppressive. We developed this technique to enable the study of solar simulated UV radiation dose responses and determined Immune Protection Factors (IPFs) for six commercially available sunscreens based on limits of protection from the dose response data. We found that the IPF did not correlate with the SPF and that protection from erythema therefore cannot be used to predict protection of the immune system. However, IPF was significantly correlated to the UVA protective capability of the sunscreens, indicating that sunscreen protection from UVA is important for prevention of immunosuppression. We recommend that sunscreens should be rated against their immune protective capability to provide a better indication of their ability to protect against skin cancer.

Key Words: Immunosuppression, contact sensitivity, Ultraviolet A, Ultraviolet B, sunscreens, human, nickel

INTRODUCTION

Ultraviolet (UV)* radiation is divided into 3 wavebands, UVC (<290 nm), UVB (290-320 nm) and UVA (320-400 nm). UVC has the highest energy, and hence is the most biologically damaging, but is blocked by the stratospheric ozone layer and does not reach the Earth's surface. Sunlight is therefore a mixture of UVB and UVA. One way UV radiation causes skin cancer is by suppressing the cutaneous immune system, impairing its ability to immunologically cancer cells [1].

Even small, suberythemal exposures to solar simulated UV radiation are immunosuppressive in humans [2]. Hence it is important that sunscreens protect not only from erythema but also from these immune effects. The few *in vivo* studies which have directly compared the erythemal and immune protective capacity of sunscreens have shown that UVA protection is an important determinant of immune protective capacity [2-4]. However, these studies used fixed-dose UV exposures rather than dose responses and hence did not determine the limits of sunscreen immune protection.

Contact hypersensitivity (CHS) to recall antigens provides a convenient and ethical model of UV immunosuppression in humans because the volunteers are already sensitised to the antigen, and multiple irradiated and unirradiated test sites can thus be evaluated simultaneously in each volunteer. Such studies can therefore be performed using much smaller numbers of volunteers than would be necessary with a primary sensitisation model. Almost 10% of the population is allergic to the nickel in their earrings, watchbands and costume jewellery. Patch testing with nickel in Finn Chambers is commonly performed in clinical practice and is a convenient and reproducible measure of UV effects on CHS responses [2].

The nickel model can be used to assess the immune effects of different UV spectra and doses. Dose responses for UV immunosuppression can be generated, and the Minimal Immune Suppressive Doses (MISD) of

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sunscreen-protected and unprotected skin determined. The ratio of these MISDs then gives the sunscreen's IPF.

MATERIALS AND METHODS

Nickel patch testing. Healthy nickel allergic volunteers were recruited for these studies. Nickel allergy was first confirmed by patch testing with Finn Chambers (Epitest, Tuusula, Finland) placed on the lower back for 48 hours. Twenty-four hours after patch removal, the intensity of nickel CHS (comprising erythema and mild induration) was measured with a reflectance erythema meter (Diastron, Hampshire, UK).

UV source. An Oriel 1000W xenon arc lamp with dichroic mirrors was filtered to produce a solar-simulated ssUV spectrum with a 7.5cm square beam (Figure 1).

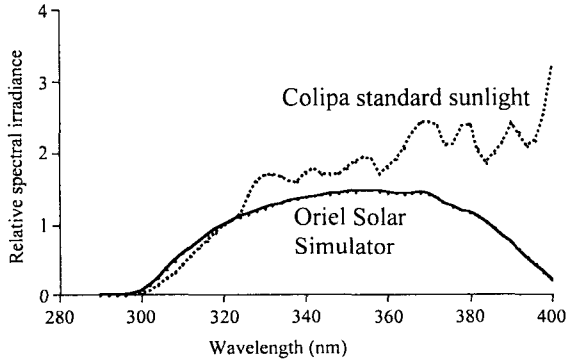

Figure 1. Spectrum emitted by the Oriel solar simulator used in these studies, compared with the standard solar spectrum (Colipa).

UV irradiation: IPF studies. Volunteers were irradiated on their lower backs through two templates daily for 4 days. Prior to irradiation, sunscreen was applied to half of the array at 2mg/cm^2 while the other half was unprotected. A range of UV doses was delivered to different areas within the templates, with the highest doses approximating the erythemal thresholds of sunscreen protected and unprotected skin. An adjacent unirradiated area of skin served as the immunologically intact control (Figure 2). Immediately after the final irradiation, nickel patches were applied to each irradiated area and also to the unirradiated control site. The patches were left *in situ* for 48 hours and the intensity of CHS measured with the erythema meter 24 hours after their removal.

Analysis of data. Immunosuppression at each test site was determined by comparing the nickel induced erythema of irradiated areas with the nickel induced erythema of the unirradiated control. Immunosuppression dose response curves for sunscreen protected and unprotected skin were determined from the pooled results from groups of 15 volunteers. The MISD was then extrapolated from these dose response curves as the UV dose causing 30% immunosuppression. We have found this to be the threshold for reproducible immunosuppression using this model. The IPF of each sunscreen was then calculated as the ratio of the MISD of protected to unprotected skin.

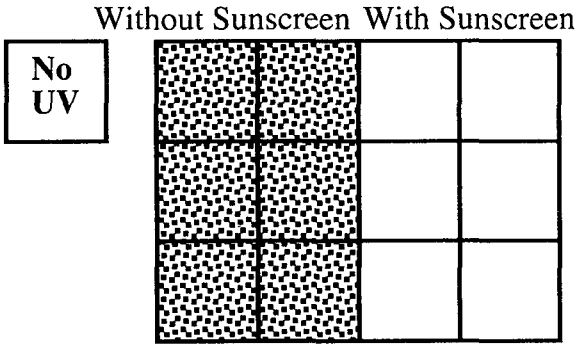

Figure 2. UV irradiation template for IPF testing. Sunscreen protected ☐ or unprotected ▩ areas were irradiated with different doses of ssUVR.

RESULTS AND DISCUSSION

UVA is immunosuppressive. In order to assess the importance of UVA to immune protection, an initial study compared 3 sunscreens with identical SPFs but differing levels of UVA protection [2]. One of the sunscreens contained cinnamate as its only active, and hence absorbed only UVB. A sunscreen containing cinnamate and oxybenzone absorbed UVB and short-wave UVA, and a third sunscreen contained cinnamate and zinc oxide (absorbing UVB and long-wave UVA). Each sunscreen-treated area, a base-lotion treated area and an untreated area were exposed to the same suberythemal dose of ssUV from a filtered fluorescent lamp array daily for 5 days. Whereas the UVB-only sunscreen failed to prevent UV immunosuppression, both of the broader spectrum sunscreens protected (Figure 3). Hence even with exposure to UV doses well below the erythemal threshold, some UVA protection is required to prevent immunosuppression.

Using the Oriel solar simulator, direct comparison of the effects of low-dose UVA and ssUV on nickel CHS demonstrated that UVA is immunosuppressive in the absence of UVB [5] (Figure 4). Three daily exposures to the amount of UVA contained in about 0.8 average minimal erythema doses of ssUV caused mean immunosuppression of almost 30%.

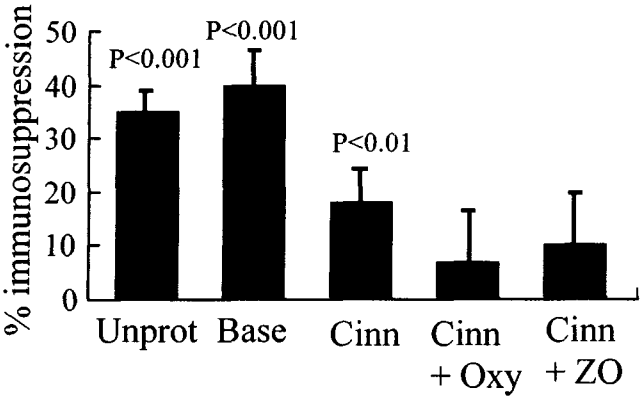

Figure 3. A group of 16 volunteers was irradiated with 97.5 mJ/cm^2 UVB and 1.23 J/cm^2 UVA from fluorescent lamps daily for 5 days before elicitation of nickel CHS. Different areas of skin were unprotected (unprot), treated with base lotion (base), or protected with sunscreens containing cinnamate (Cinn), cinnamate and oxybenzone (Cinn + Oxy), or cinnamate and zinc oxide (Cinn + ZO). The mean levels of immunosuppression are shown.

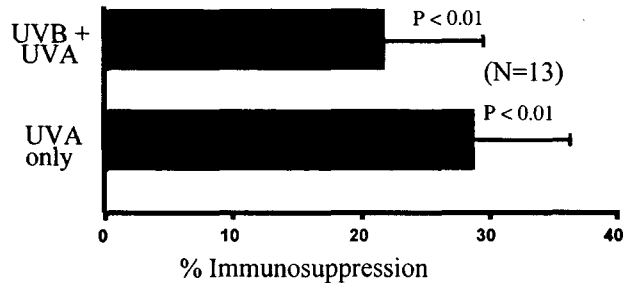

Figure 4. Three low-dose daily exposures to ssUV (UVB+UVA) and to UVA only caused immunosuppression of approximately 22% and 28% respectively.

IPF testing. We have used the nickel method to measure sunscreen IPFs. Groups of 15 nickel allergic volunteers were used to assess the immune protection conferred by 6 commercially available sunscreens. *In vitro* sunscreen absorption spectra were obtained using a Labsphere UV-1000 analyser (North Sutton, NH, USA) The *in vivo* sunscreen SPFs were determined on a separate group of non-nickel allergic volunteers using the same UV source.

There is currently no internationally accepted method for determining the UVA protective capability of a sunscreen. Two proposed methods are the Diffey critical wavelength [6] and the Boots UVA:UVB ratio. Using the *in vitro* sunscreen absorbance data, we calculated sunscreen UVA protection using both of these methods (Figure 5).

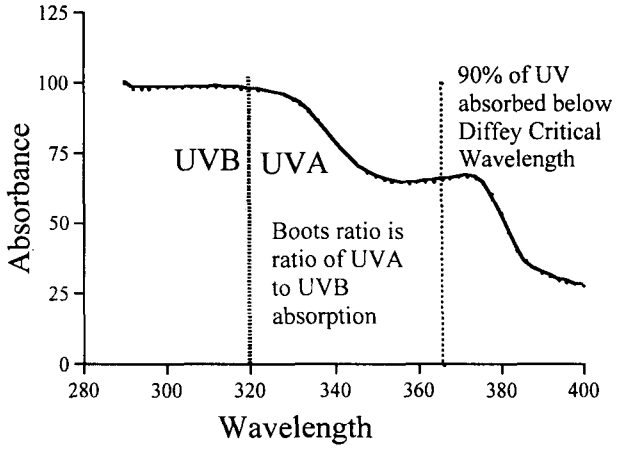

Figure 5. Example of an i*n vitro* absorption curve, as obtained for each sunscreen. UVA protection was determined using both the Diffey critical wavelength and Boots UVA:UVB ratio.

Dose response curves for immunosuppression were obtained by exposing each volunteer to 6 different doses of ssUV daily for 4 days. The MISD was then extrapolated from these dose response curves as the UV dose causing 30% immunosuppression (Figure 6). The IPF of each sunscreen was then calculated as the ratio of the MISDs of protected and unprotected skin.

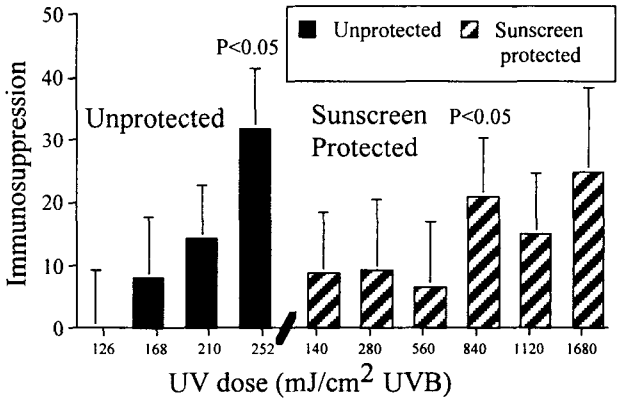

Figure 6. Dose response curves were generated for both sunscreen protected and unprotected skin using groups of 15 volunteers for each sunscreen.

We found that the IPF could not be predicted by the *in vivo* SPF of the sunscreen. There was no significant correlation when the *in vivo* SPF was plotted against the IPF results for each sunscreen (Figure 7).

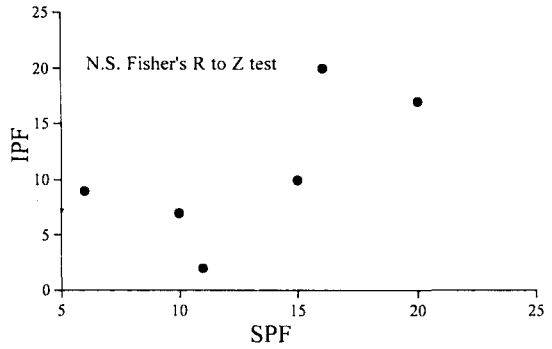

Figure 7. There was no significant correlation between *in vivo* SPF and IPF.

The IPFs were then plotted against UVA protection factors of each sunscreen as determined from the *in vitro* analysis. There were significant correlations between both IPF and the Diffey critical wavelength and IPF and the Boots UVA:UVB ratio (Figure 8). This indicates that the level of sunscreen immune protection is dependent on the level of sunscreen UVA protection, and confirms that UVA plays an important role in cutaneous immunosuppression.

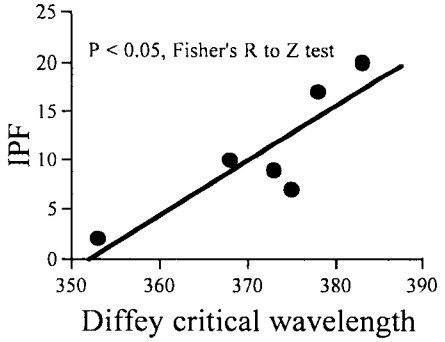

Figure 8. The Diffey critical wavelength, as determined from *in vitro* sunscreen analysis, is a proposed method for measuring sunscreen UVA protection. There was a significant correlation between critical wavelength and IPF. A similar result was obtained when IPF was plotted against the Boots UVA:UVB ratio.

CONCLUSION

We have demonstrated that UVA is immunosuppressive, both by directly examining the effects of UVA on nickel CHS reactions and by assessing the contribution of UVA protection to the immune protection conferred by sunscreens. The greater the protection from both short and long-wave UVA, the greater the protection from UV immunosuppression. Although SPF is currently the only internationally accepted parameter of sunscreen efficacy, protection from erythema does not predict protection from UV immunosuppression, and hence is unlikely to predict protection from the development of premalignant and malignant skin lesions. Sunscreens should thus provide protection not only from UVB, but also from short and long wavelength UVA as well. Whilst SPF is a useful means of ranking sunscreens for their ability to prevent UV induced erythema, there is also a need for a recognised and reproducible system for labelling the extent of sunscreen UVA protection.

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