
Improved Protection Against Solar-Simulated Radiation-Induced Immunosuppression by a Sunscreen with Enhanced Ultraviolet A Protection

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Ultraviolet radiation-induced immunosuppression is thought to play a part in skin cancer. Several studies have indicated that sunscreens that are designed to protect against erythema failed to give comparable protection against ultraviolet radiation-induced immunosuppression. One possible reason for this discrepancy is inadequate ultraviolet A protection. This study evaluated the level of immunoprotection in mice afforded by two broad-spectrum sunscreens with the same sun protection factor, but with different ultraviolet A protection factors. Both sunscreens contained the same ultraviolet B and ultraviolet A filters, in the same vehicle, but at different concentrations. Solar simulated radiation dose-response curves for erythema, edema, and systemic suppression of contact hypersensitivity were generated and used to derive protection factors for each end-point. The results of three different techniques for determining immune protection factor were compared. A

comparison of the two sunscreens showed that the protection factor for erythema in mice was similar to that determined in humans (sun protection factor) but the protection factor for edema in mice was lower. Both sunscreens protected against suppression of contact hypersensitivity but the product with the higher ultraviolet A-protection factor showed significantly greater protection. The three techniques for determining immunoprotection gave very similar results for a given sunscreen, but immune protection factor was always lower than sun protection factor. These data suggest that sun protection factor may not predict the ability of sunscreens to protect the immune system and that a measure of ultraviolet A protection may also be necessary. Key words: contact hypersensitivity/immune protection factors/persistent pigment darkening/solar simulator. *J Invest Dermatol* 114:620-627, 2000

Animal studies have shown that ultraviolet radiation (UVR)-induced suppression of cell-mediated immunity plays an important part in nonmelanoma skin cancer and a similar role is suspected in humans (Nishigori *et al*, 1996). Sunscreen use is widely advocated to reduce skin cancer risk, so it is important to know if a given reduction of erythemogenic UVR by a sunscreen is associated with a comparable reduction of skin cancer related photodamage. UVR-induced suppression of cell-mediated immunity can be evaluated *in vivo* by measuring the impairment of the contact hypersensitivity response (CHS) to chemical haptens in

mouse and humans (Noonan *et al*, 1981; De Fabo and Noonan, 1983; Cooper *et al*, 1992; LeVee *et al*, 1997; Kelly *et al*, 1998, 2000). This end-point has been widely used to evaluate sunscreen immunoprotection and make comparisons with protection from inflammation (erythema/edema). Comparisons of a given sunscreen's protection efficacies against immunosuppression and inflammation, however, have given conflicting results (Reeve *et al*, 1991; Ho *et al*, 1992; Bestak *et al*, 1995; Wolf *et al*, 1993; Roberts and Beasley, 1995, 1997; Whitmore and Morison, 1995; Serre *et al*, 1997; Hayag *et al*, 1997; Moyal *et al*, 1997; Walker and Young, 1997). Poor immunoprotection, in comparison with protection from inflammation, has been reported in the majority of these studies and this has raised doubts about the benefits of sunscreen use in the prevention of skin cancer. In many cases, however, the discrepancies between protection from inflammation and immunosuppression may be attributed, in part, to experimental design flaws. For example, very few studies have assessed immunoprotection with experimental conditions comparable with those recommended for sun protection factor (SPF) testing. SPF, a measure of acute protection from erythema, is highly dependent on the UVR source and the sunscreen's application density (Farr and Diffey, 1985) but gives no indication of a product's protection against chronic exposure. Furthermore (as shown in the results), products with the same SPF may have quite different spectral profiles. It is therefore essential to determine SPF and immune

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Abbreviations: BMDM, butylmethoxydibenzoylmethane; DNFB, dinitrofluorobenzene; EdPF, edema protection factor; EryPF, erythema protection factor; IPF, immune protection factor; IPF₅₀, 50% immune protection factor; IPF_G, global immune protection factor; ISD₅₀, 50% immunosuppressive dose; MED, minimal erythema dose; MED_D, minimal edema dose; MISD, minimal immunosuppressive dose; OC, octocrylene; PPD, persistent pigment darkening; SPF, sun protection factor; UVA-PF, UVA protection factor.

protection factor (IPF) using similar experimental conditions. Roberts and Beasley (1995) demonstrated that IPF was 2-fold greater than SPF when a solar simulator was used and sunscreens were applied at 2 mg per cm², but IPF was lower than SPF with nonsolar sources, or at a lower sunscreen application density.

Repeated UVR exposure has a cumulative effect on suppression of CHS in the mouse (Noonan *et al.*, 1981), yet most investigators determine immunoprotection using multiple rather than single exposures (Reeve *et al.*, 1991; Ho *et al.*, 1992; Bestak *et al.*, 1995; Roberts and Beasley, 1995, 1997; Whitmore and Morison, 1995; Hayag *et al.*, 1997; Moyal *et al.*, 1997). Discrepancies may also arise because of differing methods in the calculation of IPF. Bestak *et al.* (1995), who found that IPF was lower than SPF, used the ratio obtained from the highest UVR dose at which the sunscreen protected from immunosuppression vs. the minimal immunosuppressive dose (MISD). Roberts and Beasley (1995, 1997) used the ratio of UVR doses causing approximately 50% inhibition of CHS response with and without sunscreen, and found IPF higher than SPF. Walker and Young (1997) used the ratio of UVB (311 nm) doses where the CHS response was at 50% of maximal, and compared dose-response curves, with and without sunscreen. To date there have been no attempts to compare these approaches and to assess the best method of IPF determination.

Several studies have demonstrated that broad-spectrum sunscreens (290–400 nm) afford better protection than preparations that absorb mainly in the UVB region (Bestak *et al.*, 1995; Damian *et al.*, 1997; Gueniche and Fournier, 1997; Moyal *et al.*, 1997), but no studies have specifically compared different levels of UVA protection.

In order to answer some of these questions we have compared the immunoprotective efficacy of two broad-spectrum sunscreens with the same sun protection factor, but with quite different UVA protection factors (UVA-PF). We have used methods based on those recommended for sunscreen SPF testing. UVR-induced immunosuppression was assessed in hairless mice by the inhibition of the systemic CHS response to dinitrofluorobenzene (DNFB) after a single exposure to solar-simulated radiation (SSR). In addition, we have compared three methods of assessing IPF. Unlike several published studies, we have determined UVR dose-responses for all end-points with and without sunscreens.

MATERIALS AND METHODS

Animals Outbred pathogen-free female Skh-1/hairless albino mice, aged 8–10 wk were housed individually with free access to food (standard laboratory mouse pellets) and water, in a room with controlled temperature (23°C ± 2) and relative humidity (50% ± 20) and a 12 h on/12 h off light-dark cycle room lighting provided with gold lamps.

UVR sources and dosimetry The solar simulator used for all mouse studies was a 1000 W xenon arc lamp including a dichroic mirror (Oriel, Stratford, USA) equipped with a WG320/1 mm thick filter and a UG11 filter/1 mm thick filter (Schott, Clichy, France). This filtered source provided a simulated solar UVR spectrum (290–400 nm) that almost eliminated all visible and infrared radiation. Irradiance was routinely measured before each exposure session with a Centra ARCC 1600 radiometer (Osram, Berlin, Germany). The integrated irradiance, measured before the beginning of the experiment with a calibrated Bentham DM150 double monochromator spectroradiometer (Bentham, Reading, U.K.), was 2.16 mW per cm² for UVB (290–320 nm) and 16 mW per cm² for UVA (320–400 nm) at skin level.

For the determination of SPF and UVA-PF in humans, a Multiport solar simulator, Model 601 (Solar Light, Philadelphia, PA) was used. This simulator included a 150 W Xenon lamp and a dichroic mirror. It was fitted with the same Schott filters as those used in the mouse experiments, except that the WG320/1 mm thick filter was replaced with a WG335/3 mm thick filter (Schott, Clichy, France) for UVA-PF determinations. The emission beam of this simulator is focused and passed through six liquid light guides. The integrated irradiances measured spectroradiometrically at the skin level were 6.6 mW per cm² for UVB and 55 mW per cm² for UVA with WG320/1 mm filter and 50 mW per cm² of UVA with WG335/

3 mm filter. The emission spectra of the SSR sources and of the UVA source are shown in Fig 1.

Sunscreens, and SPF and UVA-PF determination Two prototype preparations (5-A and 5-B) were formulated in the same oil-in-water vehicle. Both contained octocrylene, a UVB absorber (OC or Uvinul® N539, BASF Ludwigshafen, Germany) and butyl methoxydibenzoylmethane, a UVA absorber (BMDM or Parsol 1789®, Givaudan Roure, Vernier Geneva, Switzerland) but at different concentrations as shown in Table I.

The UVR transmission spectra (T) of the sunscreen products were obtained using a modified Diffey and Robson (1989) method. In this method the UVR transmitted through a roughened quartz plate (instead of Transpore® tape as in the original method), with and without the sunscreen applied (1 mg per cm², instead of 1.5 or 2 μl per cm² in the original method), was measured spectroradiometrically and the monochromatic protection factors (mPF) were calculated (mPF = 1/T).

SPF were determined on 10 human volunteers (two skin type II, four skin type II, four skin type III) following the European Cosmetic Toiletry and Perfumery Association (COLIPA) recommendations (1994). UVA-PF was determined on eight additional volunteers (six skin type II, two skin type III) using the persistent pigment darkening (PPD) method. This technique is based on the minimal PPD dose and has been adopted by the Japanese Cosmetic Industry Association (JCIA) in 1996. PPD, first described by Hauser (1938), is the stabilized brownish-gray skin discoloration that follows the immediate pigment darkening response at about 2 h postirradiation. The minimal PPD dose of unprotected skin ranges from 15 to 25 J per cm² of UVA with a mean value of about 20 J per cm². The UVA-PF is the ratio of doses needed to obtain the minimal 2 h PPD reaction with and without sunscreen (Chardon *et al.*, 1997).

SPF were also determined in the mouse, using a modification of the human protocol, with the same SSR source used in the CHS studies. Animals were lightly anesthetized and covered with a masking template with four openings. The mean minimal erythema dose (MED), that produced a uniform pale pink color at 24 h after a single SSR exposure, was

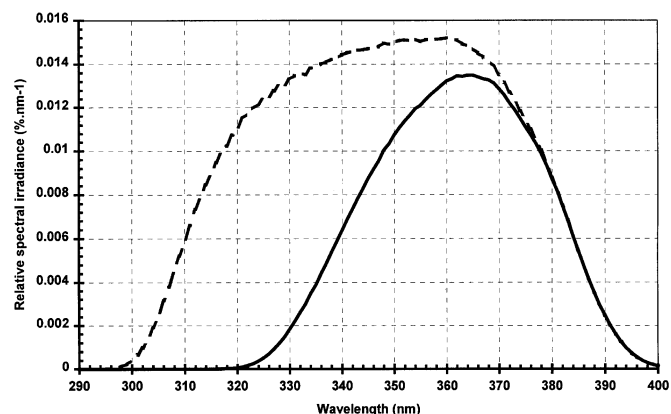


Figure 1. The emission spectra of the xenon sources comply with recognized standards. The SSR spectrum (---) meets the COLIPA standard for SPF testing. Spectra for human and mouse studies were superimposable. The UVA spectrum (—) meets the JCIA standard for UVA-PF determinations in humans. The spectra were measured with an increment step of 1 nm and are shown in relative units (the spectra were divided by the SSR total irradiance).

Table I. Characteristics of sunscreens and human *in vivo* protection factors

Sunscreen	UVB filter OC ^a	UVA filter BMDM ^b	SPF ^c	UVA-PF ^c
Product 5-A (high UVA protection)	7%	3%	7.1 ± 1.2	7.8 ± 0.5
Product 5-B (low UVA protection)	10%	0.5%	8.2 ± 2.5	3.1 ± 0.6

^aOC, octocrylene.

^bBMDM, butyl methoxydibenzoylmethane.

^cMean ± SD.

determined to be 3.0J per cm² (total SSR spectrum) using a standard protocol. One-hundred microliters of product was applied by gloved finger massage over the dorsal and flank regions (approximately 40 cm² resulting in an application density of about 2.5 mg per cm²) 15 min prior to SSR exposure. Use of templates allowed four SSR exposure doses per mouse. These were in 1 MED (group mean) increments ranging from 4 to 9 MED. SPF were determined for each product using at least 10 mice. SPF was the ratio of MED with and without sunscreen.

Irradiation protocol for CHS studies Mice were lightly anesthetized, covered with a black masking template with one opening (3 cm × 1.5 cm) over the dorsal skin area. All other body sites (i.e., ears, tail) were protected. Sunscreen products, or vehicle, were applied to the irradiated site 15 min before a single SSR exposure as described for SPF testing.

Mice were exposed to increasing SSR doses (0.5 MED increments) between 0.5 MED and 3 MED for untreated animals, 1.5 and 2.5 MED for vehicle treated animals and 4–16 MED (with 2 MED increments) for sunscreen-treated mice. Control groups included untreated unexposed mice (absolute controls), or mice treated with sunscreens/vehicle but not SSR exposed.

Measurement of inflammatory response in the mouse During the CHS studies, the inflammatory response was also assessed by erythema and edema evaluation 24 h after the single SSR exposures. Erythema was graded with a standard scale ranging from 0 (no erythema) to 4 (very intense erythema) with a value of 1 for the reaction corresponding to 1 MED. The MED was the dose that produced a uniform pale pink color with clearly defined borders 24 h postirradiation. Edema was assessed by measuring the skin-fold thickness (mean of three different dorsal sites) of each mouse with a spring-loaded micrometer accurate to 0.01 mm (Käfler, Germany). The mean dorsal skin-fold thickness for each mouse and for each experimental group was calculated and the minimal edema dose (MED_D) statistically determined. This was the lowest dose at which the skin-fold thickness became significantly greater than that of unirradiated untreated controls as detailed in the statistical methods section.

Contact sensitization protocol Five days after SSR exposure, each group of 20 mice was divided in two subgroups of 10. The mice were either treated with 50 μl of acetone or sensitized with 50 μl acetone containing 0.3% vol/vol DNFB (Sigma, St Louis, MO) on nonirradiated ventral skin. The application was repeated 24 h later. Six days after the last ventral application, 5 μl of acetone was applied to the left ear and 5 μl of acetone containing 0.2% vol/vol DNFB to the right ear of each mouse.

Twenty-four hours after this challenge, the thickness of left (acetone treated) ear and right (DNFB challenged) ear were measured by micrometer on lightly anesthetized animals. The difference in ear thickness between right and left ears represented the CHS response, expressed in mm × 10⁻², for each mouse.

The percentage of suppression of CHS response was determined for each SSR-treated mouse using the following equation and averaged for each irradiated group

$$\% \text{ suppression} = [1 - (ES_{\text{ssr}}/ES_{\text{con}})] \times 100$$

where ES_{ssr} is the individual ear swelling (elicitation) response for SSR treated (± topical treatment) DNFB sensitized mice and ES_{con} is the mean ear swelling (elicitation) response for non-SSR and nontopically treated but sensitized mice.

For a given treatment, the MISD was defined as the dose at which the mean ear swelling became significantly different (p < 0.05) from that of unirradiated unexposed sensitized controls. The dose that induced 50% suppression of CHS response (ISD₅₀), compared with the control response, was assessed graphically from the dose-response curves.

Determination of protection factors for SSR-induced inflammation and inhibition of CHS

In addition to the mouse and human SPF and human UVA-PF, five different protection factors were determined from the dose-response curves generated from the mouse CHS experiments. These were: (i) the erythema protection factor (EryPF) obtained by exposing each mouse of a given group to a single SSR dose (unlike the SPF determination already described in which each mouse receives a series of SSR doses via a template); (ii) the edema protection factor (EdPF); (iii) the immune protection factor (IPF) that is based on MISD ratio with and without sunscreen; (iv) the 50% immune protection factor (IPF₅₀) that is based on ISD₅₀ ratio with and without sunscreen; and (v) the global immune protection factor (IPF_G). A global protection factor is not based on a specific level of biologic response, e.g., minimal or 50% maximal, but instead is a measure of protection across the entire SSR dose-response range.

Statistical methods Human SPF and UVA-PF were compared by a nonparametric Wilcoxon signed ranks test. Mouse SPF were compared by a nonparametric Wilcoxon-Mann-Whitney test. For each response of the CHS study (i.e., erythema, edema, and suppression of CHS), variance-covariance analysis, with treatment as the factor and SSR dose as covariate, was used to compare the dose-response results. Analysis of variance, followed by Tukey's (1994) procedure, was performed to compare means between groups. The Dunnett's (1955) test was used to determine the minimum significant difference between sensitized unexposed untreated groups and sensitized exposed groups, for ear swelling. Then, the associated MISD observed for each treatment, were graphically determined from the respective dose-response curves of CHS inhibition. IPF was thus estimated. For each treatment, from the curve representing CHS inhibition as a function of SSR dose, ISD₅₀ was assessed graphically as the dose inducing a 50% inhibition of CHS of control group. IPF₅₀ was then calculated, as the ratio of ISD₅₀. In addition, nonlinear regressions were generated to determine global protection factors from the dose-response curves.

EryPF and EdPF were estimated assuming a global model:

$$y = f[D/PF]$$

in which y is the level of erythema or edema response at D, a given SSR dose, PF is EryPF or EdPF (with a value of 1 for the nonsunscreen group), and f a linear function fitting the dose-response curves. IPF_G was calculated similarly from the overall SSR dose range using the same global model, in which y is the percentage CHS inhibition, PF is IPF_G, with a value of 1 for the nonsunscreen group and f a sigmoidal function. Further details of this calculation are given in the Appendix.

All tests and comparisons were performed at a 5% two-sided significance level. All these analyses were performed using SAS software release 6.12 (SAS Institute Inc., Cary, NC).

RESULTS

The two broad-spectrum sunscreens have the same SPF but display different UVA-PF Tables I and II show that the SPF of the two products were very similar (7–8) in humans (p = 0.07) or mice (p = 0.6). The level of UVA protection was different (p = 0.01) by a factor of 2.5, however, as expected from the absorption profiles of the products (Fig 2).

Sunscreens offer different protection against inhibition of CHS response to DNFB but protection against inflammation is similar All SSR-exposed groups showed a significant (p < 0.05) SSR dose-response for erythema and edema (Fig 3). The mean MED was 3.0J per cm² (0.36 ± 0.02J per cm² of UVB + 2.62 ± 0.10J per cm² of UVA) and required 2 min 40 s exposure. The MED_D, equivalent to 1.4 MED in untreated mice (determined with Dunnett's 1955 test) represented an increase in dorsal skin-fold thickness of at least 6.67 mm × 10⁻² (about 10%

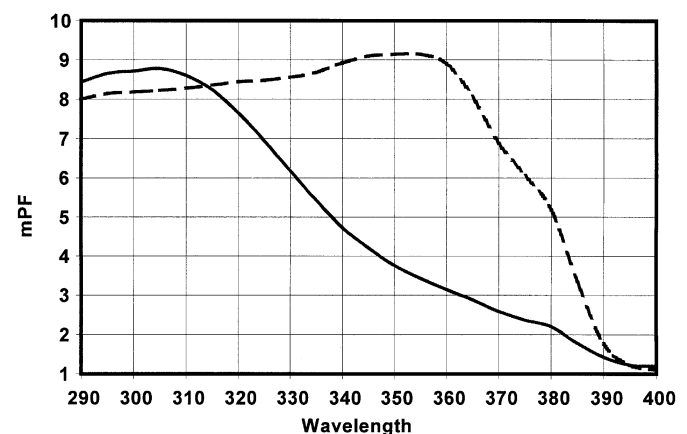


Figure 2. Two broad-spectrum sunscreens with similar SPF show differences in UVA absorption. The mPF spectra of products 5-A (---) and 5-B (—) were generated by spectroradiometric measurements between 290 and 400 nm according to a modified Diffey method (Diffey and Robson, 1989). The sunscreens were applied to roughened quartz plates at 1 mg per cm². These spectra clearly show that the efficacy of product 5-A is much higher than 5-B in the UVA range.

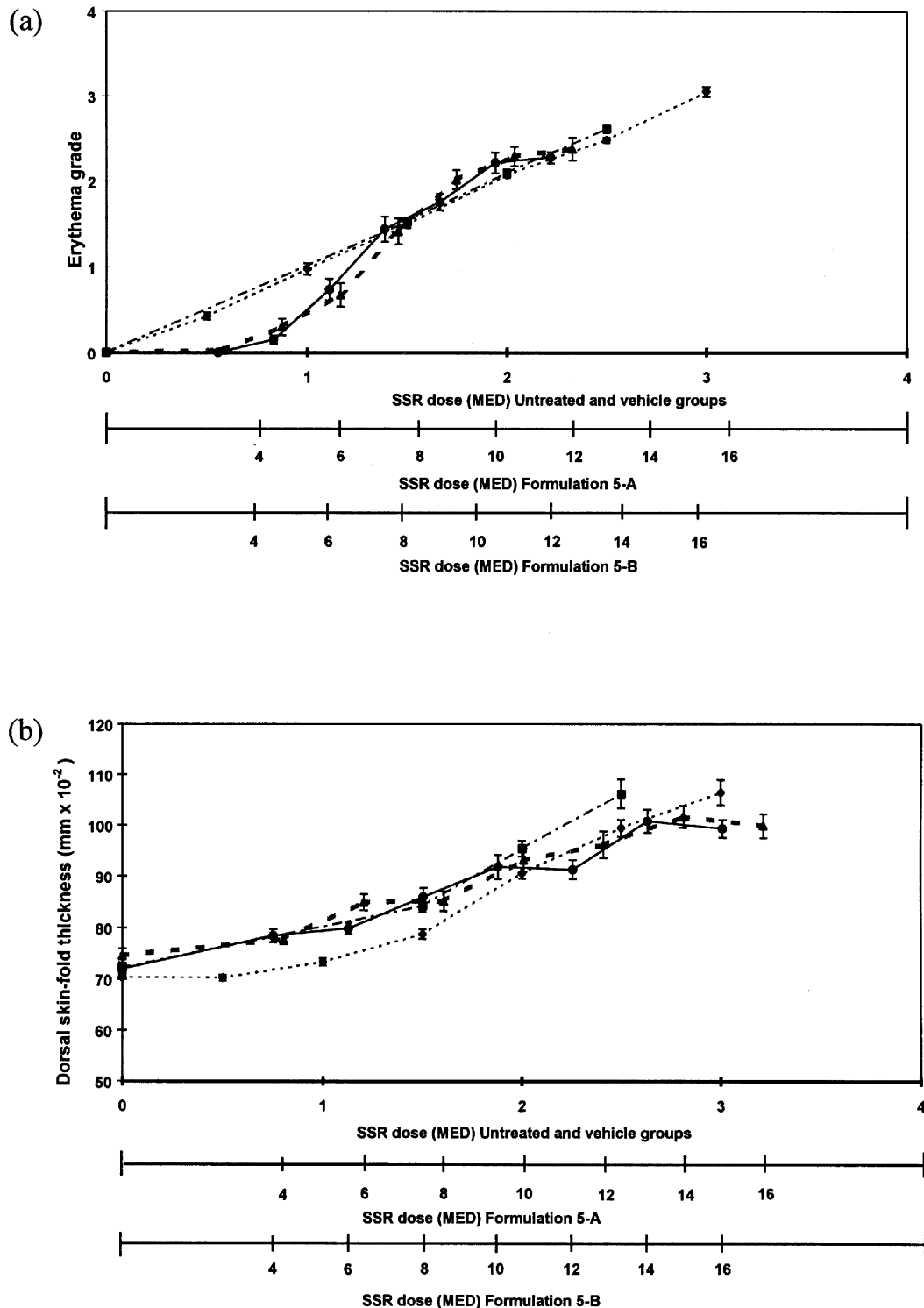


Figure 3. Two broad-spectrum sunscreens (SPF 7–8) afford comparable protection from erythema and edema over the SSR dose range tested (4–16 MED). There was a significant ($p < 0.05$) SSR dose-dependent increase in the two inflammation endpoints. (a) The dose–response curves for erythema in the different experimental groups are superimposed when EryPF is 6.9 for sunscreen 5-A and 7.2 for sunscreen 5-B. (b) For edema curves superimpose when EdPF is 5 for sunscreen 5-A and 5.3 for sunscreen 5-B. The vehicle had no effect on erythema or edema when compared with the untreated groups. No pretreatment (....), vehicle (---), sunscreen 5-A (---), and sunscreen 5-B (—). Data are given as mean \pm SEM.

more than control) (Fig 3). Variance–covariance analysis confirmed that both sunscreens products do not offer different levels of protection against inflammation ($p = 0.26$ for erythema, and $p = 0.07$ for edema).

As shown in Fig 4, suppression of CHS increased with SSR dose ($p < 0.05$). For the sunscreen treated groups (Fig 4) the dose–

response curves are sigmoid. We assumed a similar pattern for untreated or vehicle-treated exposed groups, although the plateau was not reached. Sunscreen application had no effect on the CHS response of unexposed animals. Statistical analysis of the dose–response curves of the two sunscreen-treated groups showed that the efficacy against CHS inhibition was SSR dose ($p < 0.01$) and

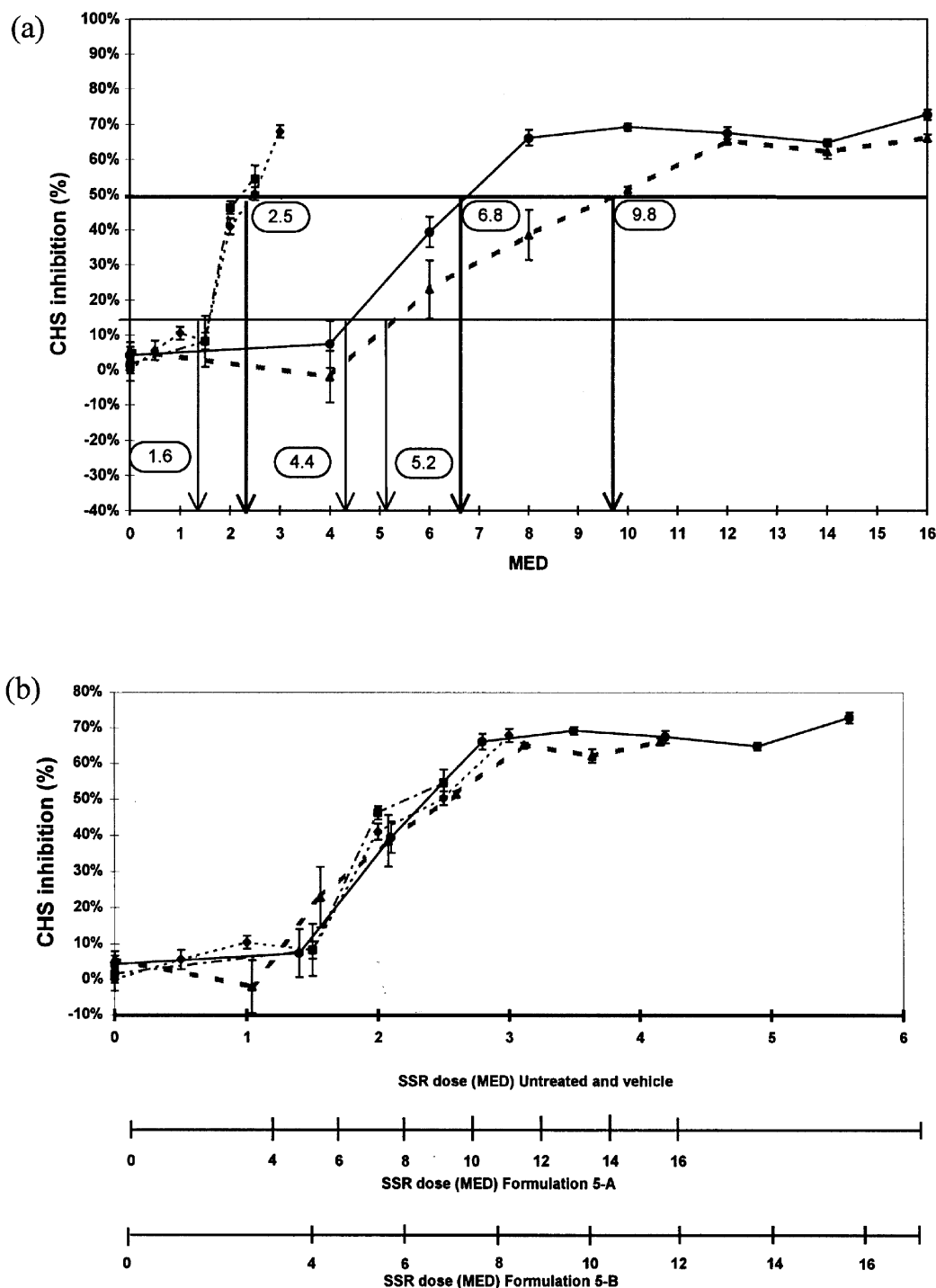


Figure 4. The sunscreen with the higher UVA-PF affords better immunoprotection. SSR dose-response for inhibition of CHS are shown; no pretreatment (....), vehicle (-.-.-.-), sunscreen 5-A (- - -) and sunscreen 5-B (—). Data are given as mean \pm SEM. (a) The immunoprotection afforded by product 5-A was significantly ($p < 0.01$) higher than that obtained with product 5-B. The IPF and the IPF₅₀ were obtained with the estimated MISD or ISD₅₀ and PF are shown in **Table II**. (b) The dose-response curves for the inhibition of CHS in the different experimental groups (with and without sunscreens) are superimposed when IPF is to be 3.9 for sunscreen 5-A and 2.9 for sunscreen 5-B. These factors named IPF_G were estimated as described in the *Materials and Methods*.

treatment dependent ($p < 0.01$) with a nonsignificant dose-treatment interaction. The globally estimated IPF offered by product 5-A (3.9 ± 0.1) was higher ($p < 0.01$) than by product 5-B (2.9 ± 0.1). Both sunscreens offered complete protection against immunosuppression at 4 MED but differed in efficacy at higher doses. Using the Dunnett's (1955) test, it was found that the minimal significant ($p < 0.05$) change in ear swelling between

exposed and unexposed animals was 3.19×10^{-2} mm. As the mean ear swelling response measured in the sensitized unexposed controls was 22.45×10^{-2} mm, the above difference corresponded to 14% inhibition of CHS response. Such inhibition was induced in untreated animals by exposing to 1.6 MED which was the MISD for this group (**Fig 4**). Using the same **Fig 4**, the ISD₅₀ for untreated animals was 2.5 MED. The vehicle had no significant

Table II. SPF, and other protection factors determined in mice from the dose-response curves for erythema, edema, and immunosuppression

Sunscreen	SPF ^a	EryPF ^b	EdPF ^b	IPF ^b	IPF ₅₀ ^c	IPF _G ^b
Product 5-A (high UVA protection)	7.3 ± 1.0	6.9 ± 0.2	5.0 ± 0.2	3.3	3.9	3.9 ± 0.1
Product 5-B (low UVA protection)	7.8 ± 0.4	7.2 ± 0.2	5.3 ± 0.2	2.8	2.7	2.9 ± 0.1

^aMean ± SD.^b± asymptotic standard error.^cGraphically determined.

effect on inflammatory and CHS responses of exposed or unexposed animals.

Protection factors of sunscreens vary with biologic endpoint assessed Globally estimated EryPF was 6.9 for product 5-A and 7.2 for product 5-B, in agreement with the SPF determined in humans and mice (**Tables I and II**). Globally estimated EdPF was 5.0 for product 5-A and 5.3 for product 5-B. These values are lower than the SPF and EryPF (**Tables I and II**). For IPF, the line corresponding to 14% CHS inhibition (inhibition induced by 1.6 MED which is the MISD for the untreated group) cut the dose-response curve of sunscreens (**Fig 4**) at 5.2 MED for product 5-A (high UVA-PF) and at 4.4 MED for product 5-B (low UVA-PF). So the IPF (based on MISD) was 3.3 (5.2 ÷ 1.6) for 5-A and 2.8 (4.4 ÷ 1.6) for 5-B. For IPF₅₀, the line corresponding to 50% CHS inhibition, cut the dose-response curves (**Fig 4**) at 2.5 MED for the untreated group, at 9.8 MED for product 5-A and 6.8 MED for product 5-B. So the IPF₅₀ were 3.9 and 2.7, respectively. The IPF_G calculated from the overall UVR dose range was found equal to 3.9 for 5-A and 2.9 for 5-B. These IPF are lower than human SPF and murine SPF, EryPF, and EdPF (**Tables I and II**). **Table II** also shows that the three IPF for each product are very similar and that better protection (by about 30%) against immunosuppression is obtained with the sunscreen with the higher UVA-PF.

DISCUSSION

We compared the protection of two sunscreens with similar SPF (7–8) against acute inflammation (erythema and edema) and immunosuppression (systemic suppression of the induction of CHS) by SSR in the hairless mouse. The products contained the same vehicle and UVB and UVA filters, and both were classified as broad-spectrum according to the modified Diffey (1994) method. The *in vivo* PPD method, however, showed that preparation 5-A had 2.5-fold greater UVA protection than preparation 5-B. The former absorbs in both the UVA-I and UVA-II regions whereas the latter shows virtually no absorption in the UVA-I range (**Fig 2**).

Erythema is routinely used to assess UVR-induced inflammation in human skin and MED is the basis of SPF determination. It is widely recognized that erythema/MED assessment in mouse skin is difficult. Nonetheless, two quite different methods in the mouse resulted in protection factors that were very similar to SPF obtained in humans. Edema in the Skh hairless strain is usually used as a model for human erythema (Reeve *et al*, 1991; Ho *et al*, 1992; Walker and Young, 1997) as their action spectra are similar and the UVR dose required for a minimal edema response is similar to a MED in a human skin type II/III (Cole *et al*, 1983). SSR-induced a dose-dependent increase in inflammation (erythema and edema) in all treatment groups. **Figure 3(b)** shows that the edema dose-response curves for all experiment groups can be made to superimpose with appropriate dose-scale adjustments. As shown in **Fig 3(a)**, however, this was not the case for erythema at the lower doses where the response in the sunscreen-treated groups was less intense. Evaluation of erythema in our study was semiquantitative and is therefore probably less accurate/reliable than the edema measurements, especially when low grade. EryPF and EdPF calculated from the dose-response curves clearly showed that both sunscreens gave comparable protection against

SSR-induced inflammation (**Table II**), but that the level of protection against edema was lower than that for erythema. We do not know the reason for this. We are not aware of any action spectrum studies for erythema in the Skh-1 mouse but, because both products gave comparable protection from edema, our data do not support an explanation based on a difference between erythema and edema action spectra.

Systemic inhibition of CHS was also SSR dose-dependent (**Fig 4**). We calculated IPF using three different methods; MISD ratio, ISD₅₀ ratio, and the overall dose-response using the global model approach. Irrespective the mode of calculation used, we found that IPF, for a given sunscreen, was comparable but always lower than the SPF, EryPF, or the EdPF (**Table II**). Reliance on the SPF and the EryPF would suggest that sunscreen immunoprotection is about half of that for protection against inflammation even when the sunscreen (product 5-A) shows good absorption over the whole SSR spectrum. Sunscreens prevent erythema by attenuating UVR and there is no reason to suppose that they modify immunosuppression via an interaction with the skin (Walker and Young, 1997). One possibility is that UVA prevents the immunosuppressive effects of UVB. Recently it was demonstrated that a single low dose of UVA could reverse the immunosuppressive effects of UVB exposure in the mouse (Reeve *et al*, 1998). In this case, the removal of UVA by a sunscreen would give a lower IPF than that predicted by the SPF. Our data, however, do not support this observation that would predict that the higher UVA-PF product (5-A) would have a lower IPF than the lower UVA-PF product (5B). As shown in **Table II** and in the *Results*, the IPF_G for product 5-A was highly significantly greater than product 5-B.

In contrast, if we assume that the EdPF is the better end-point to assess protection against inflammation in mice, the IPF_G remains lower than the EdPF but the difference for sunscreen 5-A (high UVA-PF = 7.8) is much less marked (3.3 and 5.0, respectively) than sunscreen 5-B (low UVA-PF = 3.1) for which the IPF_G is still about half that of the EdPF (2.9 and 5.3, respectively). This suggests broad-band protection is necessary to ensure that protection against inflammation and immunosuppression are comparable and suggests that UVA is immunosuppressive.

Apart from considerations such as dose-response, the protection factor against a given end-point will depend on the product of its action spectrum, the absorption spectrum of the sunscreen under test, and the emission spectrum of the source. The UVA protection factors of the products tested were based on the 2 h PPD that has a fairly flat action spectrum across the whole UVA range (Chardon *et al*, 1997). Action studies for CHS in the mouse do not extend beyond 320 nm (De Fabo and Noonan, 1983; Elmetts *et al*, 1985) but extrapolation from these studies would suggest declining efficacy with longer UVA wavelengths. Thus, it is not surprising that differences in protection factors for immunosuppression (IPF_G of 3.9 for product 5-A and 2.9 for product 5-B) are less marked than those for PPD (7.8 and 3.1, respectively).

The discussion above emphasizes our lack of knowledge of the role of UVA in immunosuppression, especially in terms of dose-response and spectral dependence. Mouse studies have shown that environmentally relevant levels of UVA-II (at 320 nm) suppress induction of CHS (De Fabo and Noonan, 1983; De Fabo *et al*,

1990) as does a single exposure to 1 MED UVA-I (340–400 nm) (el-Ghorr and Norval, 1999). In chronic studies, UVA exposure suppresses the induction of local but not systemic CHS (Bestak and Halliday, 1996). In humans, a single exposure of 4 MED UVA-II was highly effective at suppressing CHS induction (LeVee *et al*, 1997) but this was not seen with 3 MED UVA-I (Skov *et al*, 1997). Some workers have studied the effect of UVA on elicitation rather than the induction phase of CHS. A single low dose (4 J per cm²) suppressed this response (Damian *et al*, 1999) as did low-dose exposures over 1–3 d but continued exposure (4 d–4 wk) did not (Damian *et al*, 1999). Chronic exposure, however, suppresses the elicitation of local and systemic delayed type hypersensitivity responses (Moyal *et al*, 1997). Clearly, more work is required to determine the role of UVA on cutaneous immune function.

Two previous studies have evaluated sunscreen protection against UVR-induced suppression of CHS using methods based on those recommended for SPF testing (Roberts and Beasley, 1995, 1997). In the first study, an SPF 15 sunscreen preparation containing 7.5% octyl methoxycinnamate and 4% oxybenzone, was shown to have an IPF that was 2-fold greater than the labeled SPF (Roberts and Beasley, 1995). In the second study, two commercial waterproof sunscreens were tested (SPF 4 and SPF 8) and again the IPF of both sunscreens was at least 2-fold greater than the labeled SPF (Roberts and Beasley, 1997). It is difficult to explain the difference between our results and these studies but it should be noted that Roberts and Beasley (1995, 1997) did not confirm labeled SPF in their studies. Furthermore, in mouse studies, CHS responses are usually elicited on the ears, which we completely protected from UVR exposure by a UVR opaque covering. Roberts and Beasley (1995, 1997), however, used a sunscreen for this purpose. It is therefore possible that the UVR transmitted by the sunscreen enhanced the elicitation of CHS in their study, as previous murine studies have shown enhanced CHS responses after UVR exposure of the elicitation site (Polla *et al*, 1986; Grabbe *et al*, 1995).

In conclusion, our data show that three different ways of assessing IPF gave very comparable results. We provide indirect evidence that UVA radiation plays a part of unknown biologic significance in SSR-induced immunosuppression in the mouse and suggest that more research on the spectral dependence/interaction of immunosuppression is required. Our data also suggest that the level of immunoprotection afforded by broad-spectrum sunscreens cannot necessarily be predicted by SPF or by the level of protection from edema. We suggest that the most likely reason for this is an immunosuppressive effect of UVA. Finally, we have also introduced a statistical model to determine global protection factors that are indicative of photoprotection over an extended dose-response range. We believe that this concept may be important in the demonstration of protection, especially when effects, such as suppression of CHS in human skin, are seen with single suberythemal SSR exposures (Kelly *et al*, 2000).

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APPENDIX

Calculation of IPF_G . For a given mouse the following nonlinear regression model is assumed

$$y = f(t)$$

where y , the biologic response (% suppression of CHS), is a function of t , defined below:

$$t = \frac{D}{[1 \times \text{Ind}_{ND}] + [\text{PF}_A \times \text{Ind}_A] + [\text{PF}_B \times \text{Ind}_B] + [\text{PF}_V \times \text{Ind}_V]}$$

where:

$$D = \text{dose in MED}$$

Ind_{NT} , Ind_A , Ind_B , Ind_V are Boolean functions = 1 if the mouse was not topically treated, treated with product 5-A, treated with product 5-B or treated with vehicle, respectively, and = 0 where the mouse does not come into these categories.

PF_A , PF_B , and PF_V are the protection factors (IPF_G) for product 5-A, product 5-B and the vehicle, respectively, that are estimated from the model. The sigmoidal function is defined below with four parameters (a , b , c , d) that are estimated from the model.