Commercial Sunscreen Lotions Prevent Ultraviolet-Radiation–Induced Immune Suppression of Contact Hypersensitivity

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Ultraviolet (UV) radiation suppresses certain immunologic responses, such as contact hypersensitivity (CH). Some previous studies, using sunlamps emitting nonsolar-spectrum UV or excessive UV doses, have questioned the ability of sunscreens to prevent UV-induced immune suppression. Our study evaluated the immune protection capacities of commercial sunscreen lotions in relation to the effects of UV spectrum and dose. C3H mice were exposed to a fixed UV dose from Kodacel-filtered FS sunlamps that caused maximum Langerhans cell depletion and suppression of CH. Kodacel film blocks UV energy below 290 nm, thus eliminating immune-suppressive effects of UVC (200–290 nm) not present in sunlight. CH was equally suppressed in unprotected and placebo-lotion–treated, UV-exposed mice. Mice protected with sun protection factor (SPF)-15 and SPF-30 sunscreens mounted normal CH responses. SPF-4 and SPF-8 sunscreen-protected mice had CH responses significantly greater than those of unprotected mice. Direct effects of UV spectral differences on the immune protection value of an SPF-15 sunscreen were determined by exposing mice to UV radiation from unfiltered and Kodacel-filtered sunlamps and a 1000-W xenon lamp solar simulator (UV spectrum nearly equivalent to sunlight). The sunscreen immune protection value was 30 times the minimum immune suppression dose for the solar simulator, while being 7.5 times this dose for Kodacel-filtered and 2 times the dose for unfiltered sunlamps. These results demonstrate that commercial sunscreen lotions prevent UV-induced immune suppression at a level exceeding the labeled SPF when tested with an environmentally relevant UV source. Key words: immunity/sunblock. J Invest Dermatol 105:339–344, 1995

MATERIALS AND METHODS

Animals Female C3H/HeNHSd mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were age-matched (6–8 weeks old) at the initiation of each experiment. They were housed five per cage with free access to food and water. The institutional animal use and care committee approved all animal care protocols and experimental procedures according to the
UV Radiation Sources Three UV sources were used: unfiltered F250 sunlamps (FS), Kodacel-filtered F250 sunlamps (KFS), and a solar simulator. The FS source consisted of a bank of nine Westinghouse F250 sunlamps. To configure the KFS source, we placed a sheet of 0.005-inch-thick Kodacel TA407 collodion triacetate film filter (Kodak, Rochester, NY) below the F250 sunlamps to block UV energy below 290 nm. The solar simulator was a 1000-W ozone-free xenon arc lamp equipped with a dichroic mirror, 1-mm WG320 filter, and 1-mm UG11 filter. The UV energy spectra for these UV sources have been published [32].

In initial experiments, the dose of UV radiation delivered from KFS was calculated using a model UVX digital radiometer with a UVX-31 sensor (Ultra-Violet Products, Inc., San Gabriel, CA). This is a full-range UVB sensor with a peak response at 315 nm and one-half power points at 278 nm and 330 nm. In experiments comparing the effects of different UV sources, UV doses were automatically controlled. UV irradiance was measured by an IL-1700 radiometer (International Light, Inc., Newburyport, MA) equipped with an SED 400 detector. This detector, with a quartz wide-eye diffuser and neutral density filter, responds almost equally across the UV spectrum of 250-400 nm. The radiometer was interfaced with a computer that was programmed to deliver a specified UV dose through controlling the lamps by either turning off the power (FS and KFS) or closing a shutter to block the UV beam (solar simulator). Exposure conditions and UV doses for specific experiments are given in the table footnotes and figure legends.

UV Irradiation Procedure A 0.5-inch2 area on the back of each mouse was shaved with electric clippers, and the hair was removed completely by applying a commercial depilatory lotion (Nair; Carter Products, New York, NY). Animals rested 72 h before subsequent experimental procedures. For exposure to FS and KFS, mice were kept in their cages positioned at a distance of 17 cm below the bulbs. For solar simulator exposure, a group of five mice was transferred to a cylindrical polycarbonate holder placed on a rotating platform positioned in the center of the UV beam at 2 m from the lamp’s beam exit port. Exposure doses were timed in experiments monitored with the UVX radiometer. When UV doses were computer controlled, the radiometer’s UV detector was positioned in the solar simulator UV beam or under the FS and KFS sunlamps at the same level as the mice.

Sunscreens Ten commercial sunscreen lotions from four different manufacturers were evaluated. Letter designations used to identify the sunscreens, their labeled SPF’s, and percentage of active ingredients (if known) are as follows: lotion A, SPF-4, 3.5% octyl methoxycinnamate and 1% oxybenzone; lotion B, SPF-8, 7% octyl methoxycinnamate and 2% oxybenzone; lotion C, SPF-15, 7.5% octyl methoxycinnamate and 4% oxybenzone; lotion D, SPF-15, 8% padimate O and 4% oxybenzone; lotion E, SPF-15, octyl methoxycinnamate and oxybenzone; lotion F, SPF-15, padimate O, octyl methoxycinnamate and oxybenzone; lotion G, SPF-15, 7.5% octyl methoxycinnamate and 4% oxybenzone; lotion H, SPF-15, octyl methoxycinnamate and oxybenzone; lotion I, SPF-15, 7.5% octyl methoxycinnamate and 4.5% oxybenzone; and lotion J, SPF-30, 7.5% octyl methoxycinnamate, 5% octyl salicylate, 5% homosalate, and 4% oxybenzone. A sham control placebo lotion without sunscreen active ingredients was prepared by the product pilot laboratory at Schering-Plough HealthCare Products (Memphis, TN). Placebo and sunscreen lotions were applied 10–20 min before UV exposure in the amounts specified in the figure legends.

Immunoperoxidase Staining of Epidermal Langerhans Cells The staining procedure for identification of LA+ Langerhans cells has been published [33]. Briefly, epidermal sheets from the backs of UV-exposed and non-UV-irradiated mice were obtained by incubating skin biopsy specimens in 5 ml of 26 mM ethylenediaminetetraacetic acid in phosphate-buffered saline (PBS). The sheets were stained with a rat monoclonal antibody, MS/51/14 (American Type Culture Collection, Rockville, MD), raised against mouse LA+ Langerhans cells [44]. A section of each sheet was also incubated with rat serum diluted 1:100 in PBS (negative control). A peroxidase-conjugated goat anti-rat IgG diluted 1:50 in PBS (Cappel, Organon Teknika, Corp., Durham, NC) was used to bind tissue-bound primary rat antibody. Diaminobenzidine-nickel developing substrate (Zymed Laboratories, Inc., South San Francisco, CA) was used to localize tissue-bound peroxidase-conjugated secondary antibody, thus staining LA+ epidermal cells dark brown. LA+ epidermal cells were mounted on a glass microscope slide under a glass coverslip. LA+ cells were enumerated using an Optimax Digital Video Image Analyzer (Microvide Systems, Hollis, NH) coupled to a Zeiss Axiosplan Microscope (Carl Zeiss, Thornwood, NY) and interfaced to an IBM-XT computer. The LA+ cells in 10 microscopic fields (0.0420 mm2/field) of each section were counted at 400X magnification. Data are presented as the number of LA+ cells/mm2 of epidermis. Cell staining was not observed in the negative controls.

Evaluation of CH Reactivity The procedure for sensitizing mice to dinitrofluorobenzene (DNFB) has been published [35]. Briefly, 25 μl of 0.25% DNFB (Sigma Chemical Co., St. Louis, MO) in acetone and olive oil (4:1) was applied to the exposed back skin of the mice on 2 consecutive days after UV irradiation. Four days after sensitization, 10 μl of 0.25% DNFB was applied to the right ear of each mouse. After 24 h, the thickness of the left (unchallenged) ear and the right (DNFB-challenged) ear were measured using an engineering micrometer (Peacock SPI 21-355). Animals were sedated for ear measurements by intraperitoneal injection of 200 μl 4% chloral hydrate (Sigma Chemical Co.) in PBS. Two groups of non-UV-exposed controls were included in each experiment. Negative controls were not sensitized before DNFB ear challenge, and positive controls were DNFB sensitized and ear challenged along with the UV-exposed mice. These controls establish the minimum inflammatory and maximum CH ear-swelling responses, respectively.

DNFB-elicited ear-swelling responses, expressed in units of 10–2 mm, were determined by subtracting the thickness of the unchallenged ear from that of the challenged ear. Data are presented as the mean ± SEM ear-swelling units for each experimental group. The percentage suppression of the CH response in the different groups of UV-exposed mice was calculated using the formula: percentage suppression = [I – (Exp – Neg)]/(Pos – Neg)] × 100, where Exp is the mean ear-swelling response measured in the UV-exposed, DNFB-sensitized, and challenged mice; Pos is the mean ear-swelling response measured in the non-UV-exposed, DNFB-sensitized, and challenged positive control group; and Neg is the mean ear-swelling response measured in the non-UV-exposed, DNFB-challenged only, negative control group.

Statistical Analysis Ear-swelling response and Langerhans cell density data were analyzed by one-way analysis of variance using the DOS version of Statgraphics 4.0 software (Manugistics, Inc., Rockville, MD). Statistical significance between the experimental and control groups is inferred at p ≤ 0.05.

RESULTS

Establishing a UV-Irradiation Protocol to Induce Local Suppression of CH in C3H/Mice With KFS KFS have not been used previously to induce local-type suppression of CH in mice, i.e., the experimental model in which mice are sensitized through UV-exposed skin [36]. Therefore, a dose-response study was conducted (Table 1) to identify a sufficient UV dose to both render C3H mice unresponsive to DNFB-elicited CH reactions and to cause Langerhans cell depletion, the suggested mechanism.

Table 1. Comparison of UV Doses Delivered by KFS to Cause Langerhans Cell Depletion and Local Suppression of CH in C3H/HeN Mice

<table>
<thead>
<tr>
<th>Number of UV Exposures*</th>
<th>Langerhans Cell Density*</th>
<th>Percentage Suppression of CH†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1321 ± 25</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>217 ± 13 (84%)</td>
<td>77%</td>
</tr>
<tr>
<td>2</td>
<td>155 ± 12 (88%)</td>
<td>80%</td>
</tr>
<tr>
<td>4</td>
<td>105 ± 11 (92%)</td>
<td>77%</td>
</tr>
</tbody>
</table>

* C3H/HeN mice received 1.8 kJ/m2 UVB (as measured with the UVX radiometer) daily exposures from KFS given on 1, 2, 3, or 4 consecutive days. Control, non-UV-exposed mice (0) had their hair removed as did UV-exposed mice. Animals were sedated with chloral hydrate (see Materials and Methods), and their heads and tails were covered with aluminum foil during UV exposures.

† The percentage suppression of CH responses in UV-exposed mice compared with non-UV-exposed controls was determined by the formula given in Materials and Methods. Each treatment group consisted of five mice.
Sunscreens, relative to their labeled SPF, prevent UV-induced local immune suppression of CH in C3H mice exposed to UV radiation from KFS. C3H/HeN mice were irradiated on 2 consecutive days with KFS. Daily UV exposure doses were 1.8 kJ/m² + UVB, calculated from UVX radiometer measurements. Animals were sedated with chloral hydrate (see Materials and Methods), and their heads and tails were covered with aluminum foil during UV exposure. Placebo and sunscreen lotions were applied at 4 mg/cm² 10–20 min before UV exposure. Descriptions of the sunscreen lotions and details of the CH sensitization and elicitation protocol are given in Materials and Methods. Statistically significant differences (p ≤ 0.05) in CH responses between groups are indicated as follows: * the CH response was less than that of the positive control group (sensitized/challenged); † the CH response was less than that of the positive control group but greater than that of the unprotected UV-irradiated group (UV). All experiments were conducted with five mice per group. Data represent the combined results of three separate experiments.

Figure 1. Sunscreens, relative to their labeled SPF, prevent UV-induced local immune suppression of CH in C3H mice exposed to UV radiation from KFS. C3H/HeN mice were irradiated on 2 consecutive days with KFS. Daily UV exposure doses were 1.8 kJ/m² + UVB, calculated from UVX radiometer measurements. Animals were sedated with chloral hydrate (see Materials and Methods), and their heads and tails were covered with aluminum foil during UV exposure. Placebo and sunscreen lotions were applied at 4 mg/cm² 10–20 min before UV exposure. Descriptions of the sunscreen lotions and details of the CH sensitization and elicitation protocol are given in Materials and Methods. Statistically significant differences (p ≤ 0.05) in CH responses between groups are indicated as follows: * the CH response was less than that of the positive control group (sensitized/challenged); † the CH response was less than that of the positive control group but greater than that of the unprotected UV-irradiated group (UV). All experiments were conducted with five mice per group. Data represent the combined results of three separate experiments.

Sunscreens Prevent UV-Induced Immune Suppression

Ten sunscreens ranging from SPF-4 to SPF-30 were evaluated for their ability to block immune suppression of CH in mice exposed to KFS. All UV-irradiated animals, whether unprotected or protected with a placebo or sunscreen lotion, were exposed to two consecutive fixed daily doses of UVB. Figure 1 shows that the levels of immune suppression in the placebo-lotion–treated and the unprotected UV-exposure groups were equivalent. In comparison, mice protected by SPF-4 and SPF-8 sunscreen lotions mounted CH responses that were significantly greater than those of the unprotected and sham (placebo lotion) control groups. Animals protected with SPF-15 and SPF-30 sunscreens mounted CH responses that equaled those of the non–UV-irradiated positive control mice.

Sunscreens Applied in Amounts Specified for Human SPF Testing Prevent UV-Induced Immune Suppression

In the aforementioned experiments, the placebo and sunscreen lotions were applied at 4 mg/cm². Although this is higher than the amount recommended for sunscreen product SPF testing in humans [38], this was a relatively low application dose considering that at the time these experiments were initiated, the amount of sunscreen used in published animal studies ranged from approximately 15 to 25 mg/cm² [13–17]. In the one exception, the indicated sunscreen application dose was “approximately 2 µl/cm²” [20]. Data presented in Fig 2 address this issue by comparing the immune-protection capacities of SPF-15 sunscreen lotion C when applied at different amounts. Complete immune protection was provided to mice exposed to KFS UV radiation when sunscreen was applied at either 4 mg/cm² or 2 mg/cm². Application of sunscreen at 1 mg/cm² did not provide immune protection. These data show that when sunscreens are applied in the amount specified for human SPF testing (i.e., 2 mg/cm²), they provide immune protection.

Spectral Distribution of UV Energy Affects the Immune-Protection Efficacy of Sunscreens

The majority of previous sunscreen immune-protection studies were conducted with unfiltered FS-type sunlamps [13–15,18,20–22,24–28,30,31]. The UV spectra of these sunlamps are significantly different from that of sunlight [32]. Reported deficiencies in sunscreen immune protection are most likely associated with the use of UV sources that deliver high amounts of immune-suppressive, environmentally irrelevant UVC and UVB energy below 295 nm. A comprehensive study was thus conducted to compare the effects of UV spectral differences on the immune-protection level of SPF-15 sunscreen lotion C when applied at 2 mg/cm². Sunscreen-protected mice were exposed to increasing amounts of UV radiation delivered from FS, KFS, and the solar simulator. UV doses were based on a preliminary dose–response study that established the minimum

immuno suppression dose (MISD) for each UVR source as follows: 0.25 kJ/m² for FS, 0.90 kJ/m² for KFS, and 1.35 kJ/m² for the solar simulator. The specified MISD were for full-spectrum UVR energy of each source, as measured with the IL-1700 radiometer and SED 400 detector. The MISD was defined as the lowest UVR dose given to induce approximately 50% depression of the CH response in UV-exposed mice compared with the normal CH response in positive control mice (UV 1 X MISD). The MISD, based on full-spectrum UVR energy measurements with the IL-1700 radiometer, for each of the UVR sources is 0.25 kJ/m² for FS, 0.90 kJ/m² for KFS, and 1.35 kJ/m² for solar simulator. UV doses were automatically controlled by a computer linked to a UV-monitoring radiometer (see Materials and Methods). For a given UVR source, sunscreen-protected mice were exposed to a succession of increasing UV doses based on 2X multiples of the MISD, ranging from 2 to 60 MISD, until the observed immune suppression of CH responsiveness was statistically less than that of unprotected UV-exposed controls. Mice were not sedated during UV exposure; hence their ears and tails were protected with sunscreen. Data are presented as the percentage of the CH response for each experimental group relative to that of the positive control. The data presented in this figure are combined from separate experiments. There were five mice per group in each experiment. Critical data points in each experiment were repeated at least twice. Statistically significant differences (p < 0.05) in CH responsiveness between groups were calculated from raw data accumulated from individual experiments and are indicated as follows: * the CH response was less than that of the positive control group; † the CH response was less than that of the positive control group but greater than that of the unprotected UV-irradiated group (UV 1 X MISD); ‡ the CH response was less than that of the unprotected UV-irradiated group.

**DISCUSSION**

This study demonstrates the ability of sunscreens to prevent UV-induced immune suppression of CH responses in mice. Although it has been suggested that sunscreens are inadequate in preventing UV-induced immune suppression [13,14,20–22,25–27], our data clearly show that regardless of the combination of active ingredients tested, sunscreens prevent immune suppression in animals exposed to UV radiation with a spectral energy distribution similar to that of sunlight. These findings are consistent with those of Morison et al., who reported that an SPF-8 sunscreen provided complete protection against UV-induced suppression of CH [16] and tumor immunity [17] in mice exposed to sunlight. The estimated dose of solar UV energy that the animals received in these studies [16,17] was more than 10 times the human minimum erythema dose.

In previous studies [13–15,18,20–22,24–28,30,31], the immunosuppressive effect of UVC energy (wavelengths 200–290 nm) from unfiltered FS-type sunlamps was ignored. UVC effects cannot be disregarded when extrapolating experimental data to humans, because the biologically effective UV spectrum of sunlight does not extend below 295 nm. Lear et al. [32] have shown that UVC emitted by FS, although representing only approximately 3% of the total UV spectral energy, contributes about 10% of the effective energy to induce mouse skin edema and 11% to 16% of the effective energy to induce human erythema. Based on an immunosuppressive effectiveness spectrum derived from the Elmets et al. UVC action spectrum [39], we determined that UVC from FS contributes nearly 18% of the UV energy to induce local immune suppression of CH in C3H mice (Lear DB, Beasley DG, Giddens LD, Stanfield JW, Roberts LK; manuscript in preparation). Although Kodacel filtering eliminates the biologic effects of UVC [32], UVR energy below 290 and 295 nm from KFS not present in solar simulator or sunlight UV spectra contributes 3.5% of the UV energy to induce local immune suppression of CH in mice (Lear et al., manuscript in preparation).

Other experimental parameters contributing to the suggested ineffectiveness of sunscreens to prevent immune suppression include: (1) using noncommercial sunscreen preparations containing a single active ingredient [13,25–27], which is not representative of marketed sunscreens; (2) evaluating sunscreen-immune-protection efficacy based on unrelated photobiologic end points, e.g., mouse skin edema and erythema [20,22,25–31]; and (3) using UV exposure doses that exceed the UV-immunosuppressogenic absorption capacity of the sunscreen under investigation [13,14,19–23,25–28]. It has been concluded that no direct relation exists between UV-induced inflammation (as measured by skin edema or erythema) and immune suppression in mice [25–31]. We have reported that the minimum skin edema dose for C3H mice exposed to FS is four times greater than the MISD. More important, for solar-simulator UVR radiation, the C3H mouse minimum skin edema dose exceeded the MISD by more than 50-fold. Therefore, using UV doses based on skin edema or erythema as the end point to evaluate sunscreen immune protection, as done by several investigators [20,22,25–31], significantly understimates the true immune-protection capacity of sunscreens. Relying on skin edema, erythema, or other parameters, some studies [13,14,20,22,25–28,30,31] used relatively high UV doses from FS, speculated as having minimum immune suppression potential, while in fact the doses were three- to 20-fold higher than a human minimum erythema FS exposure dose [40]. Fisher et al. [20] reported that commercial SPF-6 and SPF-15 sunscreen lotions containing padimate O and oxybenzene did not prevent systemic-type immune suppression of CH in Skh-1 hairless mice exposed to five consecutive daily erythogenic doses (0.347 kJ/m²) of FS UV radiation. Likewise, the SPF-6 sunscreen did not block the induction of systemic-type immune suppression of CH in Skh-1 hairless mice exposed to five consecutive daily full UV spectral erythogenic doses (72 kJ/m²) of solar-simulator UV

† Learn DB, Beasley DG, Giddens LD, Stanfield JW, Roberts LK: Ultraviolet radiation (UVR) doses required for induction of murine skin edema and immune suppression are different and are dependent on the emission spectrum of the UVR source (abstract). *Photo­dermatol Photomed Photobiol 10:87, 1994; Lear et al, manuscript in preparation.*
to obtain accurate assessments of sunscreen efficacy.

We thank Mr. Richard Roberts for preparing the placebo sunscreen lotion evaluated in this study. We also thank Dr. Patricia Agin, Dr. Laura Crane, Dr. Doug Lern, Mr. Richard Roberts, and Mr. Joseph Stanfield, Schering-Plough HealthCare Products, for their constructive comments regarding this study. We especially appreciate the helpful discussions and comments of Dr. Curtis A. Cole, Johnson and Johnson CPI, Skillman, NJ, regarding dosimetry for discrimination and comparison of UV responses in hairless mice and humans. We are also grateful to Ms. Carolyn J. Bailey, who assisted in preparation of the manuscript.

REFERENCES


