The Study on the Ultraviolet-B Blocking Effect of Sunscreens in the Epidermal Langerhans Cells of Hairless Mice

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Background: Sunscreens have been used widely to prevent the photosensitive skin diseases, skin cancer, and skin aging. However, no sunscreen blocks all kinds of effects caused by ultraviolet light (UVL), and the effect of sunscreens on the impairment of immune function by UVL irradiation is controversial.

Objective: We try to evaluate the efficiency of sunscreens for blocking the depletion of LC induced by UVB irradiation.

Method: The ATPase positive LCs were observed in the skin of hairless mice (Hr+/Kud) irradiated by UVB with or without topical application of sunscreens. Two commercially available sunscreens with respective SPF 8 and SPF 30 were applied to the dorsal trunk skin. The mice were irradiated with different increasing doses of UVB at a single time.

Results: The ATPase positive LCs in the irradiated dorsal and ear skin were significantly decreased in densities according to the dosage, and apparently revealed a loss of their dendrites, granulation, and clumping from a UVB dose of more than 60mJ/Cm². With both sunscreen treatment on the dorsal trunk before irradiation, the densities of LCs on the dorsal skin were significantly higher compared to the un-treated groups at all ranges of UVB doses in spite of a dose dependent decrease in their density. However there was no significant difference on their preventive effect between both sunscreens (SPF 8 and SPF 30) except at high UVB doses of more than 240mJ/Cm².

Conclusion: The LC depletion induced by UVB can be partially protected through the topical application of a sunscreen at a UVB dose dependent fashion. However SPF (sun protective factor) dose not appear to be a good indicator for evaluating sunscreens immunologically. (Ann Dermatol 7(4):288~294, 1995)

Key Words: Langerhans cell, Sunscreens, UVB irradiation,

It is well known that ultraviolet light induce a number of effects on the biologic system. It is the region between 280 and 320 nm that has been found to be primarily responsible for sunburn, melanization, photoaging and skin tumor. Recent industrial development have caused an increase of air pollution such as chlorofluorocarbone (CFCs), which destroys the ozone layer in the stratosphere. As ozone molecules in the stratosphere are depleted, more ultraviolet B(UVB) passes through and a higher UVB intensity is present on the earth's surface. Consequently the interest in sunscreens is growing in this country, and hence has heightened the scrutiny of their sun protection effect. Commercially available sunscreens have been
shown to inhibit the development of UV-induced alterations in mammalian skin. For example, sunscreens containing para-aminobenzoic acid (PABA) or its analogues that absorb radiation in the UVB prevent the development of UV-induced erythema, dermal damage, and skin tumors. However, there has been controversy ones whether sunscreens inhibit the alteration of the skin's immunologic function induced by ultraviolet irradiation.

LCs have been considered as playing a crucial role as antigens presenting cells in the induction of a positive immune response to antigens introduced through the skin. They appear to be implicated in the inhibition of contact hypersensitivity and induction of tumor susceptibility.

There have been few previous studies on the effect of sunscreens in preventing the depletion of LC. Lynch et al. have shown that UV irradiation of mice treated with a commercial PABA-based sunscreen reduced the density of ATPase+LCs 3 days following irradiation, but the LC returned to normal by day 7 of irradiation. Ho et al. demonstrated that Padimate O (octyl dimethyl para-aminobenzoate) and 2-EHMC (2-ethylhexyl P-methoxycinnamate) based sunscreens inhibit respectively the depletion of the ATPase positive LCs of skin by chronic repeated UVB irradiation for 4 weeks. The difference in the result of both previous studies may be due to their UVB treatment regimen of chronic repeated exposures.

We try to see whether there is a correlation between an irradiated UVB dose and the ability of sunscreens to protect against the depletion of ATPase+LCs induced by UVB, and the protective effects correlate with the degree of SPF measured by the manufacturer. Therefore in this study the mice were irradiated with a different single dose of UVB, and both sunscreens with respectively SPF 8 and SPF 30 were compared.

MATERIAL AND METHOD

Experimental Animal

Hairless male mice (Hr+/kud), aged between 6 weeks and 9 weeks, were ranged between 15-20g in weight. They were bred and housed at a temperature and humidity controlled standard animal room in the Chonnam University Hospital. Four animals were randomly assigned to each group according to UVB doses and sunscreens.

Sunscreens

The sunscreens were Sundown 8 with SPF 8 and Sundown 30 with SPF 30 (Johnson and Johnson Co., USA). The active ingredients are octyl-N-dimethyl-p-aminobenzoate (o-PABA) and oxybenzone in Sundown 8, and 2-ethylhexyl-P-methoxycinnamate (Parol MCX), octyl salicylate, oxybenzone, titanium dioxide in Sundown 30. These were evenly applied on the area of 2 x 3 cm² of the dorsal trunk skin at 2mg/cm², and 30 minute before UVB irradiation.

UVB source and irradiation

The light source was a Fluorescent Sunlamp (Toshiba Electric Co., Tokyo, Japan) emitting chiefly UVB ranging from 290 to 315nm wavelength. The irradiance was 2.61 x 10⁶ W/cm², measured at 30cm distance from the light source. The doses were measured by UVB LMMH06 (National Biological Co., Cleveland, Ohio, USA) attached by UVB sensor. Animals were placed in the restricted cage designed so that the dorsal back and ear were evenly exposed. The different doses in 0, 30, 60, 120, 240, 1000 mJ/cm² of UVB were irradiated at a single time.

Skin biopsy and ATPase stain

The skins of the dorsal trunk and ear were removed in 0.5 x 0.5 cm² sized areas 24 hours after irradiation and the underlying subcutaneous tissue removed by scraping. The skin pieces were adhered to a transpare tapeR (3M Co., USA) by gentle pressure and floated epidermis-side up on buffered EDTA tetrasodium salt according to the method described by Baker and Habowsky, for 2.5 hours at 37°C. Then the epidermal sheet was gently separated from the dermis by fine forceps under a dissecting stereo-microscope.

ATPase stain was done following the method of Juolin and Shelley. To describe briefly, after washing two times in cold (4°C) physiological saline, the epidermal sheet was fixed for 20 min at 4°C in a buffered cacodylated formaldehyde solution. Then the fixed tissue was washed three times in cold saline for 30 min and incubated for 15 minutes in a ATPase solution (pH 7.3) with 5% MgSO4 and 2% Pb(NO3)2 and washed two times in saline for 5 minutes. It was stained for 3 min in 5% am-
monium sulfide. After washing for 5 minutes, it was mounted dermal side-up on a slide glass with glycerin jelly. The ATPase stained cells were observed and counted at 400x by using a AO microstar microscope with a grid in the eyepiece. The density of ATPase+ LCs were expressed as an average number per 1 mm² by counting four randomly selected areas. The significance was determined by a paired t-test.

RESULT

1. Alteration of ATPase positive epidermal LCs by UVB irradiation.

The number of LCs was decreased in parallel with UVB doses from 641 ± 67 to 0 in dorsal trunk skin and from 741 ± 69 to 0 in the ear skin (p<0.01) (Table 1,2). The loss of dendrites and aggregation or granulation of cells were apparently observed from a UVB of more than 60mJ/cm² and more prominent at the higher UVB doses(Fig. 1,2).

2. The effect of sunscreens on the sunscreens treated dorsal skin against the alteration of ATPase positive LCs induced by UVB.

The mean densities of ATPase positive LCs on the groups treated by both sunscreens(SPF 8 and SPF 30) were significantly higher when compared to the untreated group in all range of UVB doses. However, the mean densities were decreased in parallel with the irradiated UVB doses. This indicates that the capacity of sunscreens to protect against UVB depleting LCs is dependent on the irradiated UVB dose. In addition, there was no significant difference between either sunscreens in the UVB dose ranged from 30mJ and 120mJ/cm². This indicates that the SPF is not related to the preventive capacity of sunscreens against LCs de-
Table 1. The number of ATPase positive Langerhans cells in the dorsal skin of hairless mice after UVB irradiation with or without application of sunscreens (SPF8 or SPF30)

<table>
<thead>
<tr>
<th>UVB doses (mJ/cm²)</th>
<th>Control</th>
<th>UVB+SPF(8)</th>
<th>UVB+SPF(30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number/mm² (Mean SD) (N=4)</td>
<td>Number/mm² (Mean SD) (N=4)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>641±67</td>
<td>682±70</td>
<td>671±59</td>
</tr>
<tr>
<td>30</td>
<td>366±52</td>
<td>616±46*</td>
<td>596±45*</td>
</tr>
<tr>
<td>60</td>
<td>339±51</td>
<td>561±51*</td>
<td>551±77*</td>
</tr>
<tr>
<td>120</td>
<td>120±35</td>
<td>425±42*</td>
<td>457±69*</td>
</tr>
<tr>
<td>240</td>
<td>23±27</td>
<td>232±39*</td>
<td>322±57*#</td>
</tr>
<tr>
<td>1000</td>
<td>0</td>
<td>88±52*</td>
<td>125±64*#</td>
</tr>
</tbody>
</table>

* : paired t-test, p<0.01, as compared with the un-treated group. #: p<0.05, between UVB+SPF(8) and UVB+SPF(30)

Table 2. The number of ATPase positive Langerhans cells in the ear skin of hairless mice after UVB irradiation with or without application of sunscreens (SPF8 or SPF30) over the back skin

<table>
<thead>
<tr>
<th>UVB doses (mJ/cm²)</th>
<th>Control</th>
<th>UVB+SPF(8)</th>
<th>UVB+SPF(30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number/mm² (Mean SD) (N=4)</td>
<td>Number/mm² (Mean SD) (N=4)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>741±68</td>
<td>733±50</td>
<td>751±59</td>
</tr>
<tr>
<td>30</td>
<td>488±58</td>
<td>546±51</td>
<td>539±73</td>
</tr>
<tr>
<td>60</td>
<td>415±41</td>
<td>455±55</td>
<td>468±55</td>
</tr>
<tr>
<td>120</td>
<td>226±52</td>
<td>311±63</td>
<td>348±65 *</td>
</tr>
<tr>
<td>240</td>
<td>101±36</td>
<td>155±94</td>
<td>191±46 *</td>
</tr>
<tr>
<td>1000</td>
<td>0</td>
<td>17±34 *</td>
<td>21±33 *</td>
</tr>
</tbody>
</table>

* : paired t-test, p<0.01, as compared with the un-treated group.

Fig. 3. Comparison between sunscreen 8 and sunscreen 30 on the depletion of LC in dorsal skin by UVB irradiation (* p<0.05 between SPF 8 and SPF 30).

density (No./mm²)

- UVB alone
- UVB+SPF(8)
- UVB+SPF(30)

Density (No./mm²)

Fig. 3. Comparison between sunscreen 8 and sunscreen 30 on the depletion of LC in dorsal skin by UVB irradiation (* p<0.05 between SPF 8 and SPF 30).

3. The effect of sunscreens on the sunscreens non-treatment ear skin against the alteration of epidermal LC induced by UVB.

On the group treated by sunscreen SPF 8, the mean densities of ATPase positive LCs between both the treated and un-treated group were not significantly different in all range of UVB doses except 1J/cm². However on the group treated with SPF 30, the densities of ATPase+ LCs were higher than those of the un-treated group in the high UVB doses of more than 240mJ/cm² (Table 1,2, p<0.05). This indicates that a sunscreen with a high SPF may be effective against the systemic depletion of LC, which is probably induced only at high doses of UVB. In addition, there was no significant difference between the two sunscreens (SPF 8 and SPF 30) in the whole range of UVB doses.

DISCUSSION

Many investigations have suggested that epidermal LCs play an important role as antigen presenting cells in immune responses to antigens introduced through the skin such as with the contact hypersensitivity and skin graft rejection. The im-
munologic function of LC is also shown by the fact that they are the only cell in the epidermis that bear Fc and C3b receptor, express Ia antigens on their surface, and are of bone marrow origin. So epidermal LCs have been widely investigated in many studies associated with the immune system as they encounter both the direct and indirect effects of UVB-irradiation on epidermal tissue.

The previous studies demonstrated that epidermal ATPase positive LC irradiated by UVB are reduced in number and revealed the morphologic changes in mouse and human skin. However, Abderer et al. explained that the depletion of LCs could be due to a loss of surface markers which were detected in histochemical or immunohisto logical stain, because the depletion of LCs in irradiated skin is not observed on an electronmicroscopic examination.

In more recent studies, Iacobelli et al. demonstrated that the alterations of LCs such as a decreased number and loss of dendrite are in proportion to UVB doses. On the LC changes in guinea pig skin irradiated by UVL, they have shown that a disturbance in distribution of surface markers occurs at low doses of UVA and UVB (1-3 MED’s minimal erythema dose); and destruction of cellular membrane ‘structural changes’ occurs at a high dose ultraviolet light. Our study also revealed that the density of LCs at the skin of the dorsal trunk and ear decreases in proportion to the UVB doses increment, and a dendrite loss and uneven staining and aggregation in ATPase stain were observed with a UVB dose of more than 60 mJ/cm².

For evaluating a sunscreen’s effectiveness, the alteration of LC and contact hypersensitivity have previously been studied in the mice which have had chronic repeated UVB irradiation. Lynch et al. demonstrated the preventive effect of sunscreens containing PABA on the depletion of the ATPase positive LC by UVB was reversible. On the other hand, Ho et al. have shown that both o-PABA and 2-EHMC respectively inhibited UVB depleting LCs on the epidermis of a hairless mouse. In a more recent study, Wolf et al. have shown that the capacity of the sunscreen to prevent an inhibition of contact hypersensitivity by UVB is depend on the irradiated UVB doses. However, as far as we know, the UVL dose response in preventing LC depletion was not determined in a previous study. Our result could support the theory that the capacity of sunscreens on the LCs depletion is also depend on the UVB dose.

Most sunscreens contain one or more UVL absorbing chemicals and contain a moisturizing base. The recent most commercial sunscreens contain UVA as well as UVB absorbing chemicals. The most common ingredients are PABA and its ester, and 2-EHMC, and benzophenone-3. PABA and its ester, and cinnamate chiefly absorb UVB ranging between 250 and 320nm. Benzophenone is a broad spectrum ultraviolet filter with peak absorption at 315nm.

The sun protection effect of sunscreens has been studied in various aspects. The alterations of various biologic events in the UVL irradiated skin such as sunburn cell production, suppression of contact hypersensitivity, epidermal ornithin decarboxylase change, tumor susceptible state, alcoactivation capacity of keratinocyte, NK cell activity, have been previously investigated to evaluate the efficacy of sunscreens.

The sun protection factor widely used by manufacturers is represented as the ratio of MED before and after application of sunscreen in the white skin ranged skin type I-III. However, the complete standardization of the usefulness of sunscreens has not been established until now, because the effectiveness of a sunscreen may be determined by safety to physical factors such as sweating and water, and by methods of measurement.

Our result demonstrated that there was no difference at preventing the depletion of LCs between SPF 8 and SPF 30 except at a high UVB dose of more than 240mJ/cm². The discrepancy between the SPF and the capacity of protective effect on the depletion of LC may be explained as the different maximum action spectrum. For determining SPF, a 150 watt high pressure xenon-arc lamp (solar simulator) that provides a spectral output similar to sunlight 290-400nm UV region is recommended as a light source. However, the maximum activity of immune suppression is related to two UV waveband. One is at 293nm, which is the peak of the action spectrum for pyrimidine dimer formation, the another is at 270nm for UV absorption and photoisomerization of the normal epidermal component urocanic acid. We presumed that both sunscreens may have the same
ability to preserve the LCs from UVB.

Immunologic impairment caused by UVB can be divided into local and systemic effects. Local immune suppression is defined as the diminished contact hypersensitivity response observed when haptenes are applied through UV-irradiated skin, and systemic suppression when haptenes are applied at a distant, non-irradiated site. The capacity of sunscreens to prevent an immunologic impairment is controversial. In previous studies by Lynch et al. and Fisher et al., the systemic suppression of contact hypersensitivity has not been achieved by PABA or PABA ester. However Morison and Wolf et al. demonstrated a partial inhibition on the suppression, and Reeve et al. revealed that the inhibition depends on the constituent of sunscreens.

On the mechanism for immune impairment, the induction of hapten specific T-suppressor cells by UVL, an impairment of antigen presenting cell function and soluble factor release from irradiated epidermal cells such as Cis-urocanic acid, prostaglandin, keratinocyte derived factors appear to be involved in immune impairment by UVL. Furthermore, Noonan et al. previously presented that the alteration of LC on the un-irradiated site didn’t occur as a systemic effect of UV radiation.

However this study revealed that the depletion of ATPase positive LC by high doses of more than 120mJ was protected in the group treated by the sunscreens with SPF 30. The reason may be explained by the following. First, an ingredient of the sunscreen cutaneously absorbed from the back skin may protect the LC depletion, because the ear skin is close to dorsal trunk. Second is the blocking of certain soluble factors released from the highly irradiated skin, which may be released only at a high dose UVB, or may be released in an enough large amount to induce the depletion of distant LCs. So we presume that the changes of LC on the un-irradiated site may be induced if a higher dose UVB is irradiated on the other site.

REFERENCES

15. Bergstresser PR, Toews GB, Sterilein JW: Natural