The Degrees of UVB-Induced Erythema and Pigmentation Correlate Linearly and Are Reduced in a Parallel Manner by Topical Anti-Inflammatory Agents

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To examine whether it is possible to evaluate the degree of ultraviolet B (UVB)-induced inflammation by measuring the degree of hyperpigmentation, we investigated the relationship between UVB-induced erythema and the subsequent pigmentation quantitatively. At 24 h and 7 d after irradiation with erythemogenic doses of UVB to the backs of 16 Japanese subjects, the degree of induced erythema (Δerythema index) and that of pigmentation (Δmelanin index) were examined by an image analytic method using a videomicroscope interfaced with a computer. The relationship between two indices was linear in each subject, and the correlation coefficient was 0.83 when evaluated using whole data. The slope of the regression line for the Δmelanin index against Δerythema index tended to become steeper as non-irradiated skin color became darker (r = 0.63), suggesting that more efficient melanogenesis takes place after the same level of inflammation in the subject with darker skin. Both erythema and hyperpigmentation were suppressed significantly and in a parallel manner by corticosteroids and indomethacin applied topically immediately after UVB irradiation. These results imply that the post-inflammatory hyperpigmentation correlates closely with the severity of the prior inflammation and that chemical mediators released in the inflammatory process have considerable influence on the melanogenesis. We conclude that the measurement of UVB-induced hyperpigmentation can be utilized for the assessment of topical anti-inflammatory agents, unless these have direct actions on the tyrosinase activity of melanocytes. ***Key words: melanogenesis/corticosteroid/indomethacin. J Invest Dermatol 103:642-646, 1994***

Because of the simplicity and good reproducibility, ultraviolet B (UVB)-induced erythema has been utilized for a model of inflammation to assess the efficacy of anti-inflammatory agents such as cyclooxygenase inhibitors [1-3], corticosteroids [3-7], and antioxidants [3,8]. The method is problematic, however, because it reflects the complex outcome of two dynamic processes, i.e., the natural time course of the erythema and the time-dependent changes in the effect of drugs. Should we apply these agents before or after UVB irradiation and when should we evaluate the results?

We noted that potent corticosteroid applied topically just after UVB irradiation reduces tanning as well as erythema. This observation has led us to speculate that postinflammatory hyperipigmentation may be another quantitative measure of inflammation and may offer several advantages for the assessment of anti-inflammatory agents in comparison with the measurement of erythema.

MATERIALS AND METHODS

Design of the Study Sixteen healthy male Japanese aged 24-38 years (mean, 28), all of whom were skin type III according to the Fitzpatrick classification [9], participated in this study. None of them had skin diseases or history of photosensitivity. At first, 12 different doses of UVB (12, 24, 36, 48, 60, 72, 84, 96, 120, 144, 192, and 240 mJ/cm^2) were administered, divided into two parallel rows, to their upper backs (area of each test site, 1 cm × 1 cm) with Dermaray (Eisai, Tokyo, Japan) with an array of four fluorescent tubes (FL20S-E, Toshiba, Tokyo, Japan). The radiation spectrum of this light source lies mainly in the range of UVB, with a peak radiation wavelength of 350 nm **(Fig 1)**. Immediately after irradiation, no immediate pigment darkening was observed at the site exposed to the maximum dose (240 mJ/cm^2). This indicated that the effect of UVA was negligible for the purposes of this study. At 24 h and 7 d after the irradiation, still video pictures of the test and control areas were obtained with a videomicroscope (Dermascope, Yayoi, Tokyo, Japan) and the extent of erythema (24 h) and pigmentation (7 d) were measured by image analysis. In addition, minimal erythemal dose (MED) and minimal tanning dose (MTD) were determined on inspection by three dermatologists.

Next, double doses of MED were irradiated on four sites, each of which was 1 cm × 1 cm in size, in a single line in the middle backs of 10 subjects. Immediately after the irradiation, 0.05% clobetasol propionate, 0.1% hydrocortisone butyrate, 1% indomethacin, and hydrophilic ointment, all of which are commercially available creams, were applied to each of the irradiated areas (0.03 ml per area) using disposable syringes. The applications were randomized to rule out regional variation in response to UV. Test sites were then covered with 1.5 × 1.5 cm plastic film and fixed with Silky pore plaster. Three hours later, all dressings were removed and residual creams were wiped off. The evaluation of erythema was carried out at 9 and 24 h after the irradiation, and that of pigmentation after 7 d.

Using the same techniques, 0.1% hydrocortisone butyrate cream was applied to three test sites on the backs of 10 different subjects immediately after the irradiation. At 1, 6, and 20 h after the application, one of the dressings, determined randomly, was removed and residual cream was carefully wiped off. The erythema and pigmentation were evaluated at 24 h and 7 d after the irradiation.

Instrument and Measuring Procedure The instrument used for the quantification of erythema and pigmentation has been described elsewhere

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Abbreviation: MTD, minimal tanning dose.

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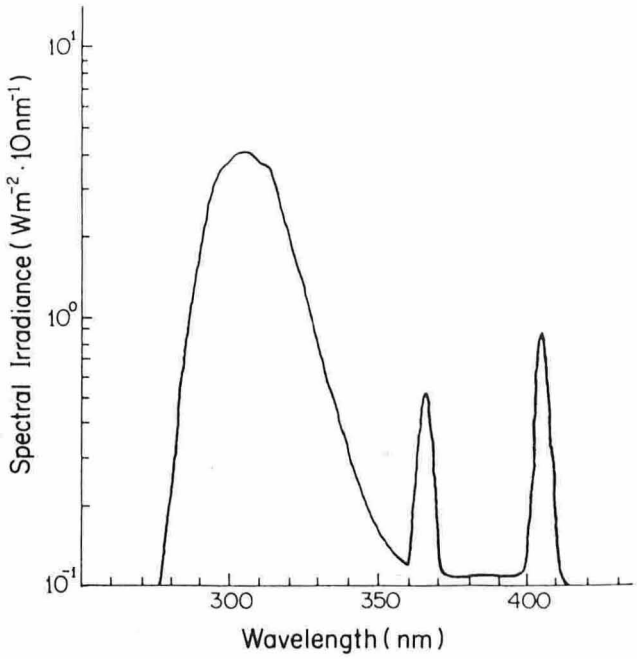

Figure 1. Spectral irradiance of the light source.

[10]. After recording of a color image of the skin (view field of the instrument, 10 × 11 mm) with a videomicroscope, we analyzed brightness of all picture elements in each band of red, green, and blue with a computer. By referring to the brightness data of a white standard, we estimated average reflectance values of the skin in each band. Erythema and melanin indices were defined as follows, based on the theories by several authors [10–13]:

$$\text{erythema index (EI)} = 100 \times [\log_{10}(1/Rg) - \log_{10}(1/Rr)];$$

$$\text{melanin index (MI)} = 100 \times \log_{10}(1/Rr),$$

where Rg and Rr are the reflectance values of the measured area in the green and red bands, respectively. The logarithm of the inverse reflectance approximates the absorbance value of the skin [11] in each band of red and green. We determined both indices for the central area (50 mm^2) of the image of a test site to prevent any influence from the peripheral darkening of the image. Instead of measuring test sites before irradiation for the control, we measured adjacent normal skin at every measurement and took the difference in the indices between test site and normal skin to avoid diurnal variation in skin color and the possible variation in the illuminance of the light source of the videomicroscope. The degree of erythema (Δerythema index) and that of pigmentation (Δmelanin index), therefore, are defined as follows:

$$\Delta\text{erythema index } (\Delta EI) = \text{EI of test site} - \text{EI of adjacent skin};$$

$$\Delta\text{melanin index } (\Delta MI) = \text{MI of test site} - \text{MI of adjacent skin}.$$

Although affected by each other to a certain extent, the erythema and melanin indices are thought to have a linear relationship with the hemoglobin content in the superficial vascular plexus and the melanin content in the epidermis, respectively [10]. However, the two indices, especially the melanin index, do not represent the actual hemoglobin and melanin content of the skin, because the values are "padded" with the light absorption by other skin components such as the dermis and stratum corneum. The Δ values, on the other hand, are capable of eliminating this "padding" and indicate the real difference in the amount of chromophores between a test site and the control.

Before every series of measurement, brightness calibration of the light source of the system was made against a white standard. All measurements were carried out in the prone position after about a 10-min rest. This study was performed in winter and the room temperature ranged from 22°C to 24°C. For statistical analysis, the Student paired t test was used in the comparison of any two data groups, and $p < 0.05$ was considered significant.

RESULTS

The results on each subject are shown in **Table I.** The skin color of each subject was defined as the averaged melanin indices of all control sites when the first series of the experiment were done.

Table I. Skin Color (averaged melanin index), MED,[a] MTD,[a] Slope of the Regression Line in the ΔMelanin and ΔErythema Indices,[b] and Correlation Coefficient (r) Between the Two Indices[b] for All Subjects

Number	Skin Color	MED	MTD	Slope	r
1	14.6	48	72	2.75	0.95
2	14.5	36	72	2.11	0.69
3	16.4	48	72	3.87	0.89
4	11.3	36	60	1.50	0.91
5	13.3	60	72	1.75	0.98
6	12.0	36	60	2.22	0.97
7	16.5	72	84	1.98	0.97
8	12.8	36	60	2.09	0.82
9	13.7	60	96	1.57	0.98
10	11.7	60	96	1.37	0.96
11	16.0	36	60	3.26	0.94
12	15.1	72	96	2.19	0.96
13	11.2	72	96	1.23	0.87
14	15.2	48	60	3.20	0.95
15	14.5	48	60	4.37	0.87
16	12.1	48	72	2.34	0.95

[a] mJ/cm^2.
[b] Test sites with no erythemal reaction are excluded.

Clearly demarcated erythema and pigmentation were not perceptible by clinical inspection until both Δerythema and Δmelanin index become higher than 1 (index value) in most subjects. The MTDs were higher than MEDs for all subjects by 12–36 mJ/cm^2. There was no significant correlation between skin color and both MED and MTD.

Figure 2 is an example of the relationship between the Δerythema index at 24 h after irradiation and the Δmelanin index after 7 d. A good linear correlation between them was found in the range above MED, and similar results were obtained in most subjects. The magnitude of the slope of the regression line in this range on each subject, which represents the extent of tanning that takes place after the same degree of erythema is induced, is also shown in **Table I.** A significant correlation ($r = 0.63$, $p < 0.01$) was observed between the skin color and the slopes of the regression lines **(Fig 3)**. The range of variation in the slopes, however, was so narrow that a good

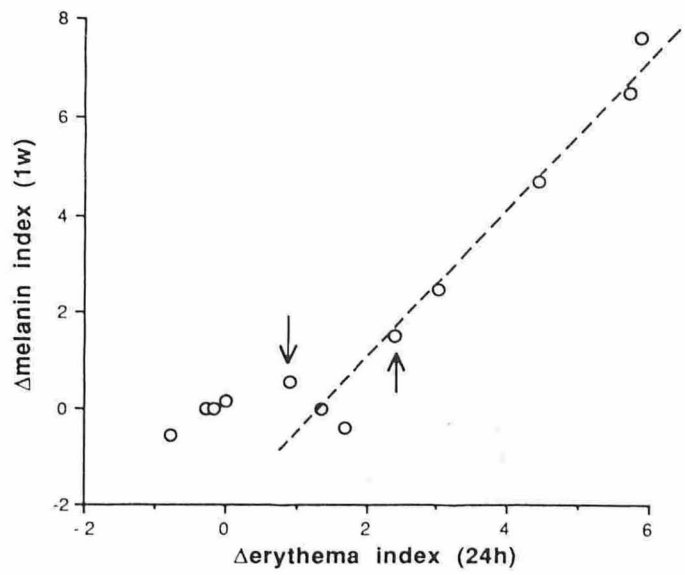

Figure 2. An example of the relationship between the degree of erythema (Δerythema index) and that of pigmentation (Δmelanin index) measured at 24 h and 7 d, respectively, after UVB irradiation. *Arrows,* minimal erythema (↓) and minimal tanning (↑) based on visual judgment; *broken line,* regression line when test sites with no erythemal reaction are excluded.

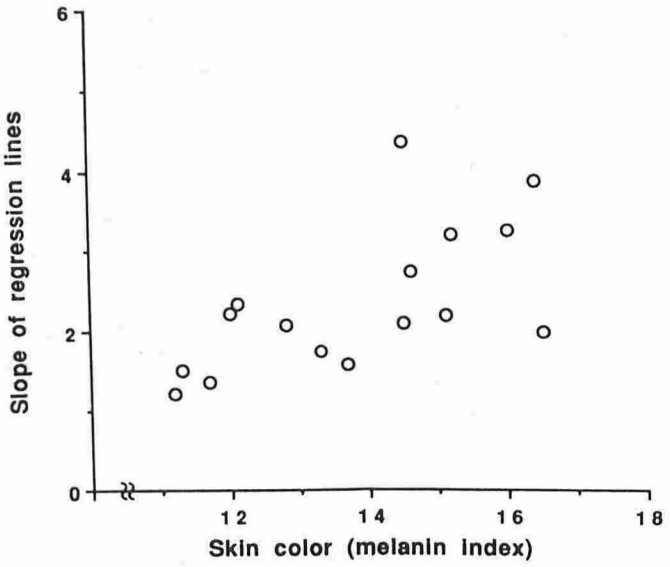

Figure 3. Positive correlation between the intrinsic skin color (averaged melanin index for non-irradiated sites) and the magnitude of the slope of the regression lines (increase in melanin index per unit increase in erythema index). Correlation coefficient: 0.63, $p < 0.01$.

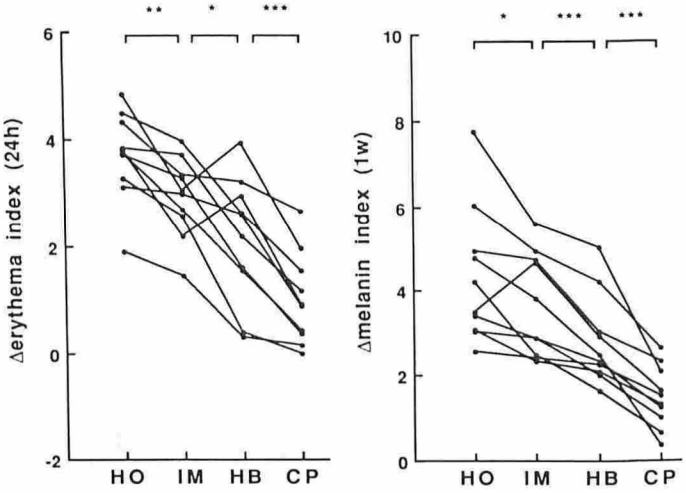

Figure 5. Both UVB-induced erythema (24 h, *left*) and pigmentation (7 d, *right*) are suppressed in a parallel manner by topical anti-inflammatory agents. The irradiation dose was 2 MED. The agents were applied for 3 h immediately after irradiation. HO, hydrophilic ointment; IM, 1% indomethacin; HB, 0.1% hydrocortisone butyrate; CP, 0.05% clobetasol propionate. The differences between any combinations of the mean values are significant. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

linear correlation ($r = 0.83$, $p < 0.001$) was noted between the Δerythema and Δmelanin indices in the range of the erythemogenic dose (≥MED) when whole subjects' data were plotted **(Fig 4)**.

Concerning the effect of the topical agents, UV-induced erythema both 9 and 24 h after the irradiation were significantly suppressed by treatment with indomethacin and two kinds of corticosteroids when compared to those treated with hydrophilic ointment **(Fig 5, *left*)**. The Δerythema indices in corticosteroid-treated sites showed negative values in large part at 9 h after irradiation (data not shown), indicating that the blanching phenomenon was stronger than erythema formation. Tanning after 7 d was also suppressed in a fashion similar to that of the erythema after 24 h **(Fig 5, *right*)**. Clobetasol propionate showed the strongest suppression, followed by hydrocortisone butyrate and indomethacin, with statistically significant differences. This result was in complete agreement with the clinical grading. A linear relationship similar to that in **Fig 4** was found between the Δerythema and Δmelanin indices **(Fig 6)**. However, the Δmelanin index (7 d) was higher than 1 (index value) for most test sites where the Δerythema index (24 h) had been lower than 2, indicating that tanning took place even at sites where erythema (24 h) had been sufficiently suppressed. This finding is distinctly different from **Fig 4** and suggests that the degree of erythema 24 h after irradiation is not necessarily crucial for that of subsequent tanning.

Figure 7 shows that the duration of the application of hydrocortisone butyrate has an influence on the erythema and tanning after

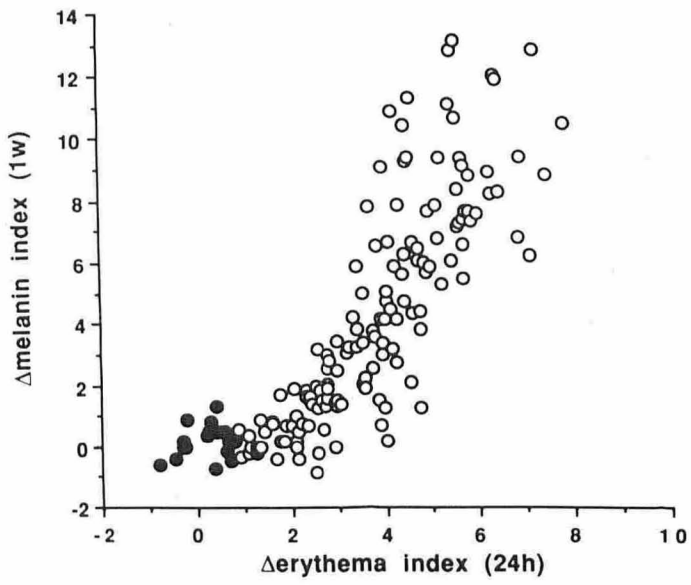

Figure 4. The degrees of UVB-induced erythema (Δerythema index 24 h) and pigmentation (Δmelanin index 7 d) correlate linearly. All subjects' data (irradiation dose ≥ 36 mJ/cm²) are plotted. Correlation coefficient is 0.83 ($p < 0.001$) when test sites with no erythema reaction (●) are excluded.

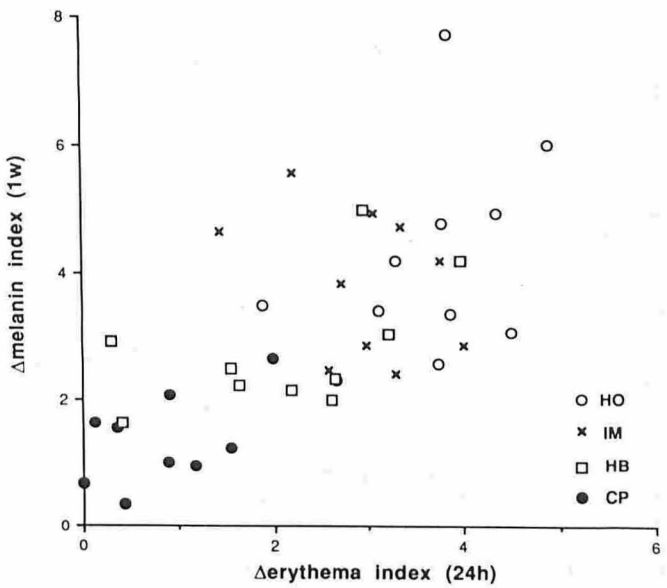

Figure 6. Relationship between the degree of the suppression of UVB-induced erythema (Δerythema index 24 h) and that of pigmentation (Δmelanin index 7 d). Experimental data and abbreviations used are identical to those in **Fig 5**.

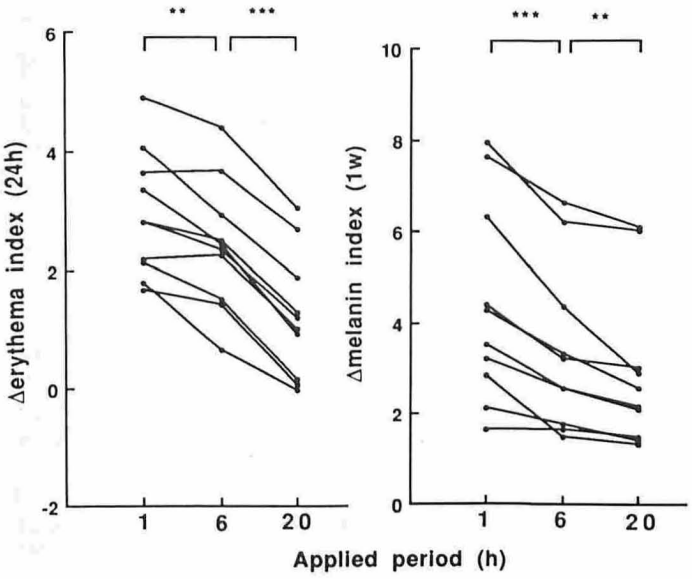

Figure 7. Both UVB-induced erythema (24 h, *left*) and pigmentation (7 d, *right*) are increasingly suppressed with an increase in the duration of topical application of 0.1% hydrocortisone butyrate. The irradiation dose was 2 MED. The differences between the mean values are all significant. *** $p < 0.001$, ** $p < 0.01$.

irradiation. There were significant differences between the mean Δerythema index of 1-h– and 6-h–treated sites, as well as between 6-h– and 20-h–treated sites at 24 h after irradiation **(Fig 7, *left*)**. A parallel result was obtained in the Δmelanin index after 7 days **(Fig 7, *right*)**, indicating that both erythema and tanning are suppressed more efficiently with increased duration of application.

DISCUSSION

Although there seems to be variation in the chemical mediators involved in UVB-induced erythema depending on species [14], arachidonate metabolites, especially prostaglandins (PGs), are known to play an important role in the induction of erythema [15–20]. Histamine is also involved in the erythema formation in guinea pigs [14] and maybe in human skin [3,18], possibly by enhancing PG synthesis in UVB-irradiated skin in the early phase of inflammation [20]. Interestingly, it has been reported that these mediators stimulate melanocytes of human [21,22], guinea pig [23], and mouse skin [24] *in vitro*. Although direct action of UVB on melanocytes is also expected to be involved in the post-irradiation tanning, these findings suggest that UVB-induced inflammation and subsequent tanning are not independent phenomena and that the intensity of inflammation may affect tanning.

The present study showed that MTD was higher than MED, ranging from 1.17 to 2 MED, in all subjects by inspection. This result can be seen clearly in **Fig 3**, in which the Δmelanin index does not change until the Δerythema index reaches around 2 (index value). In the range above this point, the degree of pigmentation (Δmelanin index) is proportionate to that of the prior erythema (Δerythema index). Because the erythema and melanin indices are expected to have linear correlations with the blood volume in the superficial vascular plexus and the melanin content in the epidermis, respectively [10], the result implies that the content of melanin produced in the post-inflammatory process correlates linearly with the extent of the hyperemia due to UVB-induced inflammation. Indomethacin and corticosteroids reduced not only erythema but also hyperpigmentation in a parallel manner. In addition, both erythema and hyperpigmentation were more suppressed when the duration of application of a corticosteroid became longer. Taken collectively, these results indicate that the degree of tanning is not determined by the irradiation dose alone, i.e., by the direct action of UVB to melanocytes, but is strongly influenced by the intensity of the prior inflammation.

To the best of our knowledge, neither indomethacin nor corticosteroids reduce the tyrosinase activity of melanocytes. Because PGs stimulate melanocytes or melanogenesis *in vitro* [21–24], it is likely that the suppression of UVB-induced pigmentation is due at least partially to the reduction in PG synthesis through the inhibition of cyclooxygenase by indomethacin and the induction of annexin or lipocortin by corticosteroids [25]. Parallel suppression of UVB-induced erythema and pigmentation can be explained with this hypothesis, especially in the case of indomethacin. However, the case with corticosteroids seems to be more complex, because skin blanching due to topical steroids is independent of PG-related anti-inflammatory activity [26]. In addition, corticosteroids might suppress the production of endothelins, which have been shown to be produced by human keratinocytes [27] and to enhance human melanogenesis [28], directly or indirectly, by suppressing the release of related cytokines from keratinocytes.

It is likely that UVB-induced pigmentation offers several advantages when utilized as a parameter of the prior inflammation. Although pigment quality is influenced by the redox reactions of melanin and degradation of melanosomes [29], UVB-induced pigment is more accurately assessed by reflectance measurement than erythema, because the former is localized in the epidermis, whereas the latter is due to vasodilatation of the capillaries and superficial plexus, which are embedded in the dermis, and thus more difficult to probe uniformly. In addition, postinflammatory pigmentation can be regarded as a more cumulative or integrated parameter of UVB-induced inflammation than erythema, because it is highly likely that the degree of pigmentation is influenced not only by that of inflammation at the peak point around 24 h after irradiation, but also by subsequent changes, and possibly by the overall inflammatory process. When utilized for the assessment of anti-inflammatory agents, however, the measurement of UVB-induced pigmentation has the following limitations and pitfalls: i) adequate irradiation doses can be different for subjects with different skin types; ii) desquamation drastically affects the result; iii) anti-inflammatory agents that affect tyrosinase activity cannot be properly evaluated; and iv) the results should not be extrapolated for measuring the hypopigmenting properties of anti-inflammatory agents. Our preliminary conclusion is that 2 MED is most adequate for the assessment using Japanese subjects, because tanning induced by lower doses was too weak to enable us to determine the difference between the action of various agents, and because higher doses often resulted in desquamation 7–14 d after irradiation. In a study using skin type II and III subjects, Andersen *et al* [30] found no significant difference in pigmentation between skin treated with three different anti-inflammatory compounds and the vehicle control 10 d after UVB irradiation, although they found a clear trend in the degree of reduced pigmentation. Judging from the results of the present study, we believe that subjects with relatively strongly pigmented skin, such as Mongoloids, are better for the assessment of tanning suppression due to anti-inflammatory agents than those with little or no pigmentation.

A significant, positive correlation between skin color (melanin index) and the slope of the increase in the Δmelanin index against that in the Δerythema index is noteworthy. This result implies that darker skin produces melanin more efficiently than fairer skin after the UVB-induced inflammation of the same intensity, suggesting that the difference in the melanin production depends on the constitutional skin color. The measurement of the magnitude of the slope therefore seems to contribute to the discrimination of the so-called skin types, as their definition is now based mainly on the clinical relation between sunburn and following suntan. If the measurement of hyperpigmentation, however, is used for the assessment of anti-inflammatory agents, it seems more appropriate if the variation in the slope is smaller among the test subjects.

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