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Intensity and Area Increase of UVB-induced Erythema: Two Variables Used for Studies of the Influence of Topically Applied Drugs

LENNART JUHLIN[1] and BRAHAM SHROOT[2]

[1]*Department of Dermatology, University of Uppsala, Uppsala, Sweden and* [2]*CIRD Galderma, Valbonne, France*

The area of UV erythema produced by a small beam head was found to increase with increasing doses. The aim was to investigate whether measurement of the area could be a more useful indicator of UV-induced damage than classic visual grading. Topical pretreatment with all-*trans* retinoic acid (tretinoin) and betamethasone valerate was used to test the applicability of the method in pharmacological studies. We used a round outlet head (5 mm²) connected by optical fibre to a monochromatic irradiator, and doses ranging from 0.05 to 0.2 Joule of 300 mm UV light were applied to the skin of 6 healthy subjects. Erythemal area was calculated by the measurement of two diameters, and intensity was graded visually (0–6 scale). The area of the erythema correlated with the increase in intensity up to score 6. Area measurement was less subject to intra-investigators' variability than the intensity score. By multiplying intensity by area, a good indicator of UV-induced reactivity was obtained. Pretreatment with betamethasone valerate decreased the area of erythema, as did tretinoin 12 h after irradiation. Thus, area measurement of erythema is a useful adjunct to visual grading of UV-induced skin reactions.

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L. Juhlin, Department of Dermatology, University Hospital, S-751 85 Uppsala, Sweden.

When previously investigating the effect of drugs on UVB-induced erythema, we observed that the area of erythema increased with higher doses (1). Here we have examined the utility and precision of measuring the area of the erythema, as opposed to simply grading the intensity from 0 to 6 by visual observation. The new approach was applied to investigation of the effect of pretreatment with betamethasone and retinoic acid before irradiation. Multiplying erythema intensity by area gives an improved measure of UV-induced reaction than intensity or area alone.

METHODS

Subjects investigated

Six healthy subjects (4 men and 2 women, age 26–34 years, sun-reactive II–III) participated in the study. They were not tanned and were not taking any drugs except contraceptive pills.

Test materials

All-*trans* retinoic acid (tretinoin) 0.01%, betamethasone valerate 0.1%, and tretinoin 0.01% + betamethasone valerate 0.1% were dissolved in polyethylene glycol (PEG) 25% and ethanol.

Treatments before irradiation

Areas of 2 × 5 cm on the inside of the forearm were washed with alcohol, marked and treated with 0.1 ml of test solutions, dried for 2 min and covered with a hydrocolloid dressing (Actiderm® Convatec-Squibb, USA), which was left on for 4 days. After removal of Actiderm the area was washed with alcohol and left open to dry for 10 min and then irradiated. A non-treated area was also irradiated.

Photoirradiation procedure

A monochromatic irradiator was used (clinical photoirradiator, Applied Photophysics Ltd, London, UK) set at 300 nm and equipped with an optical fibre (length 1 m), with a round outlet head of 5 mm² (diameter of 2.5 mm) which was pressed slightly against the skin (1). The following doses were administered on the back: 0.05, 0.1 and 0.2 Joule. The irradiation emitted was measured at each use by a built-in dosimeter.

Grading of the test site reactions

The tests were read at 12, 24 and 48 h after irradiation by 2–3 observers, who were unaware of the treatment used for each area. Each grading was performed in duplicate under similar ambient conditions of light and temperature.

Visual grading of intensity of erythema

The following scale was used: 0 = no erythema, 1 = hardly visible erythema, 2 = faint but clear erythema, 3 = moderate erythema, 4 = marked erythema, 5 = severe erythema and edema, and 6 = very severe erythema and edema.

Measurement of area (A)

As the area was generally circular, two diameters (90° angles) were measured in mm and the area was calculated as:

$$\frac{\pi(d_1 \times d_2)}{4}$$

Reactivity (R) was calculated by multiplying intensity by area (R = A × I).

Statistical evaluation

Intra- and inter-variability of investigators reading UV-irradiated area (A) and scoring intensity (I). Intra-investigator variability is determined from the readings obtained by one investigator consecutively evaluating the 15 sites on the same volunteer. Variance was estimated for the 24 and 48 h data when investigators performed the 15 evaluations repeatedly giving (15–1) × (n-1) degrees of freedom (df). There are 14 × 7 df for the 24-h period and 14 × 14 df for the 48-h period. In total there are 21 × 14 = 294 df for the intra-investigators' variance.

The inter-investigator variance was calculated from the means of the 15 readings at the 24- and 48-h time points for each investigator (with n-1 df). The degrees of freedom used for weighting were 7 for the 24-h point and 14 for the 48-h point, making a total of 21 df. Use of an analysis of variance model then allowed us to make clearer estimates more precisely.

Treatment difference. The scores were averaged for the readers and analysis of variance was applied to the resulting data at each time point and UV dose.

When the Fisher F statistic from the Anova model (2) was significant at the 0.05 probability level, pairwise comparisons of each treatment vs PEG were performed by *t*-tests using the residual variance. When the F-test was not significant no individual comparison

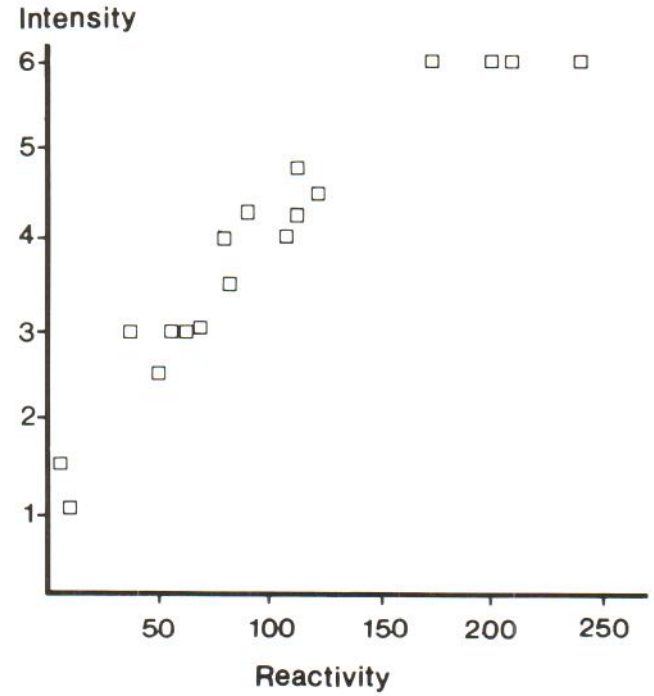

Fig. 1. Intensity (I) and reactivity (R = I × Area) 24 h after UV irradiation of vehicle-treated skin. Mean values of 2 independent observers are given.

was performed. Such pairwise comparisons by protected t-test were carried out in order to minimize the false positive rate.

RESULTS

When measuring the area the standard deviation (SD) was the same (SD = 3.1 mm^2) for inter- and intra-investigator variations. Intensity score readings were homogeneous over the entire area and the inter-investigator variance was higher (SD = 0.72) than for intra-investigators (SD = 0.57). The SD for the reactivity (R = A × I) was 21 and 18 for intra- and inter-variability respectively.

In all treatment groups there was a linear correlation between reactivity area and intensity, but at the highest intensity score only the area and reactivity continued to increase as illustrated in Fig. 1.

The skin pretreated with betamethasone valerate and betamethasone valerate + tretinoin showed 12 h after irradiation a significantly decreased area ($p < 0.05$–0.01) at all doses of UV irradiation, but the intensity score was only decreased ($p < 0.01$) at the highest dose (0.2 J) and the reactivity at 0.1 and 0.2 J ($p < 0.01$). At 12 h the tretinoin-treated skin also showed significantly decreased intensity ($p < 0.05$) and reactivity ($p < 0.01$), as illustrated in Fig. 2, but there was no significant decrease of area. Twenty-four hours after irradiation only betamethasone valerate and betamethasone valerate + tretinoin-treated sites had a decreased area ($p < 0.05$) and reactivity ($p < 0.01$) when compared to PEG alone. At this time the PEG-treated skin showed an increased reactivity when compared to the non-treated and non-occluded skin (Fig. 2).

DISCUSSION

When investigating the influence of drugs on UV-induced erythema, pretreatment with tretinoin and betamethasone valerate altered the area of the erythema (unpublished observations). The present pilot study was designed to examine the importance of measuring the area in addition to the estimation of intensity by visual examination. Our statistical evaluation indicates that area measurement is less subject to intra-investigator variability than the intensity score. The smallest area of erythema measured was 5 mm^2, which corresponds to the size of the outlet head. The maximum area observed in this study was 42 mm^2. To best detect an increase in area, the irradiated zone should be small but still large enough to judge the intensity of the erythema. This may be one reason why area changes have previously not been reported. There are two possible explanation why erythema is observed beyond the 5 mm^2 aperture of the irradiation device held against the skin. Either on reaching the skin irradiation is scattered, or more likely there is lateral diffusion of mediators causing erythema.

Schmied & Saurat (3) have previously reported that tretinoin does not affect the vasoconstrictive effect of steroids. Our results indicate that the tretinoin pretreatment does not affect

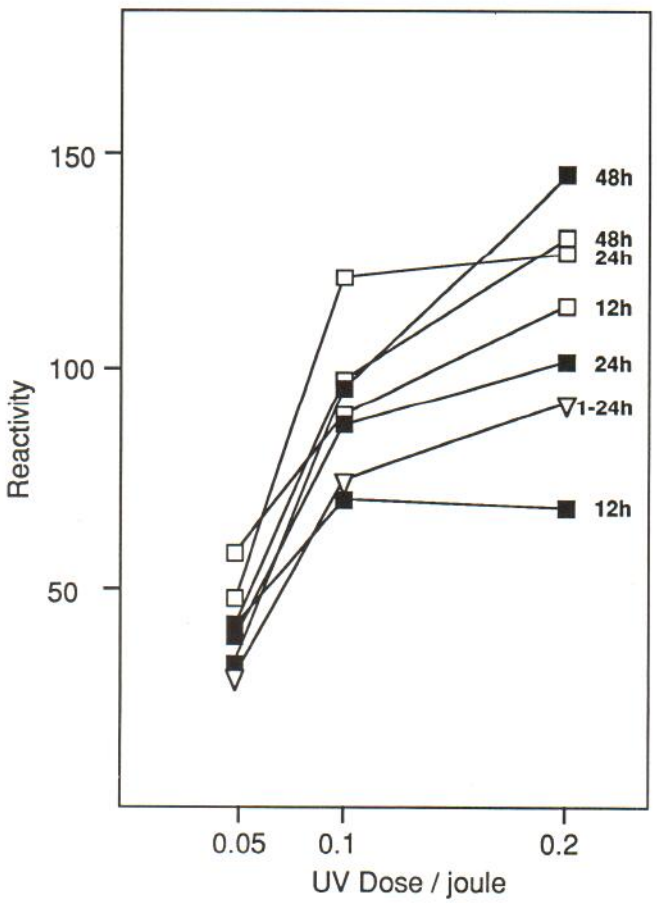

Fig. 2. Reactivity to 3 doses of UV irradiation at various times in skin pre-treated with retinoic acid, its vehicle and in non-treated skin. The mean observations in 6 healthy subjects are shown. ■-----■ tretinoin, □----□ vehicle, ▽-----▽ non-treated.

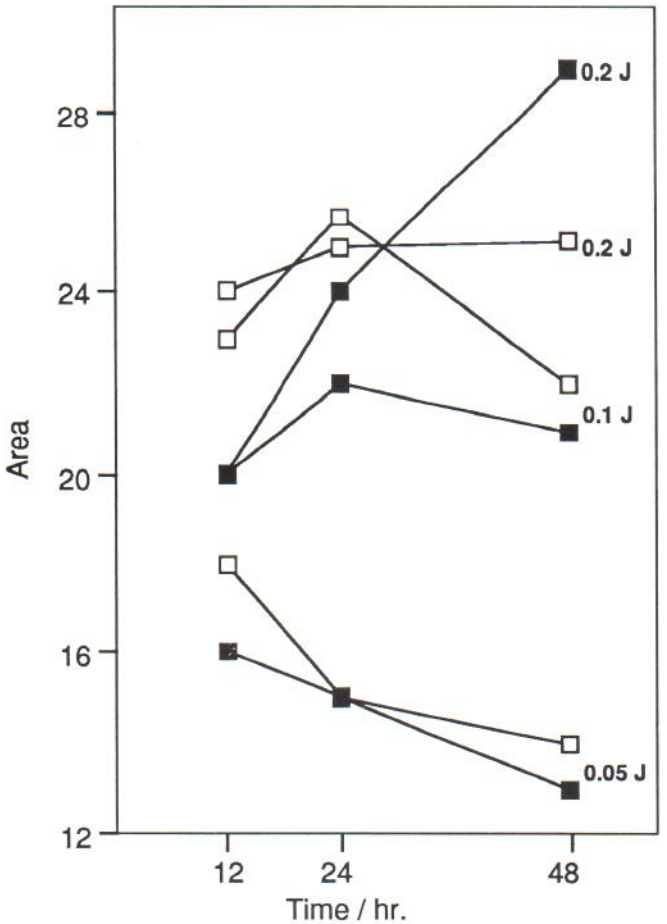

Fig. 3. Area of erythema in tretinoin and vehicle pre-treated skin 12, 24 and 48 h after irradiation. The mean observations in 6 healthy subjects are shown. ■----■ tretinoin,□----□ vehicle.

the UV erythema-inhibiting properties of betamethasone valerate. We conclude that the decrease in area resulting from betamethasone valerate pretreatment may be explained by vasoconstriction. This decrease, however, is limited to the zone adjacent to the 5 mm^2 area corresponding to the outlet head.

Fischer et al. (4) found that, compared with a vehicle-treated area, after treatment with 0.1% tretinoin in a cream base under occlusion for 4 days, the stratum corneum was more compact, the epidermis thicker, and intracellular spaces increased. These modifications might decrease the erythema in tretinoin-treated skin but cannot explain why only the reaction to 0.2 J at 12 h is decreased (Fig. 2). High stratum corneum levels of tretinoin at the 12-h period could act as a UV filter, and tretinoin may degrade in the skin and produce pro-inflammatory products by 48 h. This would also explain why at the highest dose (0.2 J), tretinoin treatment enhanced, in a time-dependent manner, the UV erythema response as judged by area measurement (Fig. 3). Since we previously reported that anti-inflammatory drugs inhibited the early phase of the UV erythema reaction at 12 h but not a late phase reaction at 48 h (1) and observed, in model systems the anti-erythema activity of tretinoin (5), we cannot exclude the possibility that tretinoin inhibits the early phase of UV erythema in man.

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