Rapid Assay of the Anti-inflammatory Activity of Topical Corticosteroids by Inhibition of a UVA-induced Neutrophil Infiltration in Hairless Mouse Skin

I. The Assay and its Sensitivity

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A single large dose of UVA induced an intense infiltration of neutrophils into the lower dermis of hairless mouse skin, peaking at 24 h. The ability of 15 name brand topical corticosteroids to suppress this infiltrate was determined. The rank order of suppression correlated with the accepted clinical category of anti-inflammatory potency. This is a rapid screening procedure for assaying the anti-inflammatory activity of new steroids and for optimizing the vehicle.

A variety of human and animal models have been developed for assessing the anti-inflammatory activity of topical corticosteroids (1–7). Vasoconstriction is a speedy and popular screening technique (1), although skin sites can vary in sensitivity to the assay (8). The other general approach has been to determine the capacity to inhibit experimentally induced inflammatory reactions in human skin, viz, contact allergy or croton oil pustules.

Human studies are troublesome for both technical and ethical reasons. Individual responses are highly variable and the tests are burdensome. Assessment of anti-inflammatory activity in murine species is feasible (9) but has not come into general use, probably because of limited sensitivity (10).

The prelude to the current work was an observation by Gilchrest et al. that 50 J/cm^2 of long-ultraviolet radiation (UVA) to human skin resulted in a modest infiltrate of neutrophils (11). Our aim was to demonstrate that irradiation of hairless albino mouse skin with a single large dose of UVA produces a massive influx of neutrophils into the dermis. This is a dramatic and quantifiable event, which forms the basis of a new model for assaying topical corticosteroids.

MATERIALS AND METHODS

Two-month-old albino female Skh-hairless-1 mice were obtained from the Skin and Cancer Hospital of Temple University Health Sciences Center, Philadelphia, Pa. Unrestrained mice were irradiated in treatment groups of two with a single dose of 195 J/cm^2 of long wavelength UVA. This dose is approximately 3 UVA minimal erythema doses for the hairless mouse. The UVA lamp was a UVASUN 3000 (Mutzhas Productions; Munich, Germany), which emits a continuous spectrum of long wavelength ultraviolet radiation with a sharp cut-off of radiation below 340 nm (12). Irradiance was monitored with an IL 700A Research Radiometer (International Light, Inc., Newburyport, Mass,

USA) using a sensor with peak sensitivity at 360 nm. At the level of the animal's dorsum, 60 cm below the source, irradiance was 15 mW/cm^2.

Time course of neutrophilic response

Dorsal trunk skin was excised immediately and 5, 10. 16, 24 and 42 h after irradiation. The specimens (1.5 × 0.5 cm) were fixed in formalin, processed for light microscopy and stained with hematoxylin and eosin.

Corticosteroid assay

100 µl of 15 name brand corticosteroids were evenly applied to dorsal trunk skin immediately post-irradiation and again 12 h later. Petrolatum was used as a control. Biopsies were taken at 24 h after irradiation when the density of neutrophils was at a peak. The number of neutrophils in the dermis was counted in ten fields per specimen under oil immersion (magnification 1000×). The mean value ± the standard error of the mean, per field, was then calculated. The microscopist was blind regarding the identity of the test steroids.

RESULTS

Time course

Fig. 1 depicts the neutrophilic responses at various intervals
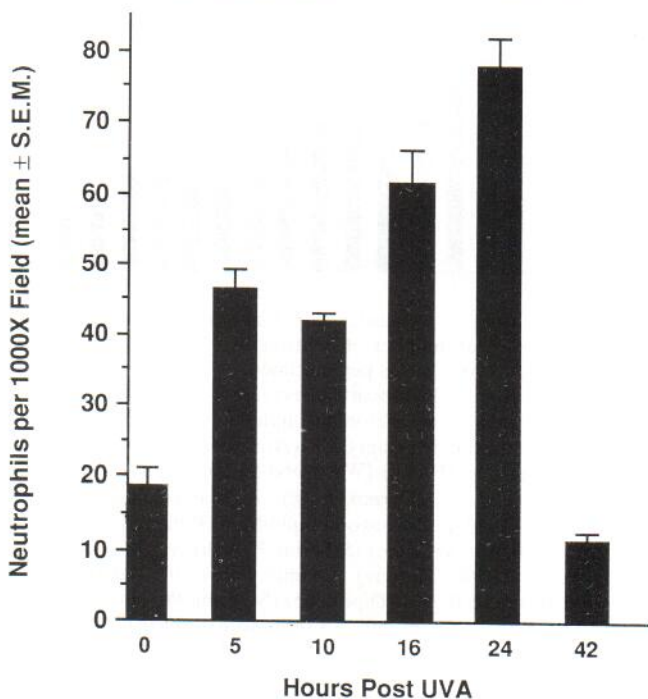

Fig. 1. Time course of the neutrophilic response. Using two animals per time point, the mean number of neutrophils (± S.E.M.) was determined from ten 1000× fields per specimen.

Fig. 2. Hairless mouse skin. (Hematoxylin and eosin ×255). A. UVA-irradiated (195 J/cm²): petrolatum control. There is a profuse neutrophilic infiltrate accompanied by severe edema which displaces collagen bundles, ruptures lipocytes and separates muscle bundles in the panniculus carnosus (→). B. Normal skin showing sparse cellularity, dense collagen, intact lipocytes (*) and a coherent panniculus carnosus (→).

over 42 h. In unirradiated mice there were 1–2 neutrophils per high power field. Immediately after irradiation the density of neutrophils averaged 19 per field. A doubling of this occurred by 5 h. The density reached a peak at 24 h and had largely resolved by 42 h. At 24 h the infiltrate was greatest in the deep

Fig. 3. Rank order of corticosteroid potency. Using two animals per treatment, the mean number of neutrophils (± S.E.M.) was determined from ten 1000× fields per specimen.
Key: 1. Petrolatum, 2. Hydrocortisone cream 0.5% (Hytone) (Dermik Labs). 3. Triamcinolone acetonide ointment 0.1% (Aristocort), 4. Triamcinolone acetonide cream 0.1% (Aristocort) (Lederle). 5. Halcinonide cream 0.1% (Halog) (Westwood-Squibb). 6. Hydrocortisone cream 2.5% (Hytone) (Dermik Labs). 7. Triamcinolone acetonide cream 0.5% (Kenalog) (Westwood-Squibb). 8. Betamethasone valerate ointment 0.1% (Valisone) (Schering-Plough). 9. Diflorasone diacetate cream 0.05% (Florone) (Dermik Labs). 10. Betamethasone dipropionate cream 0.05% (Diprolene) (Schering-Plough). 11. Clobetasol-17-propionate cream 0.05% (Termovate) (Glaxo). 12. Diflorasone diacetate ointment 0.05% (Florone) (Dermik Labs). 13. Betamethasone dipropionate ointment 0.05% (Diprolene) (Schering-Plough). 14. Betamethasone valerate cream 0.1% (Valisone) (Schering-Plough). 15. Clobetasol-17-propionate ointment 0.05% (Termovate) (Glaxo). 16. Fluocinolone acetonide ointment 0.025% (Synalar) (Syntex). 17. No UVA, no topical treatment.

dermis, extending even into the muscle layer (panniculus carnosus) (Fig. 2a). This was accompanied by edematous swelling of the tissue that often ruptured lipocytes. By contrast, Fig. 2b shows normal unirradiated skin, no inflammatory infiltrate and intact tissue.
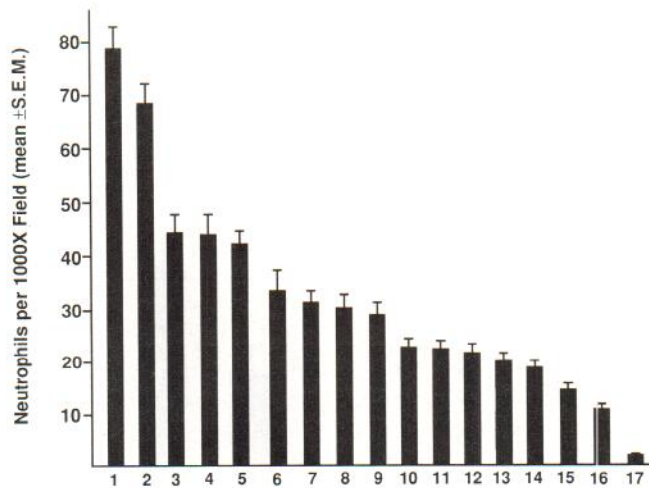
Corticosteroid assay

Fig. 3 compares the anti-inflammatory activities of 15 name brand topical corticosteroids. The rank order of potency correlated well with the known anti-inflammatory potency of these steroids.

DISCUSSION

Human anti-inflammatory screens based on suppression of induced inflammatory reactions are onerous. With the large number of corticosteroid preparations now available, a simple method to rank corticosteroids is desirable. In vitro models would be irrelevant since the innumerable interactions involved in the inflammatory cascade cannot be duplicated ex vivo. Other means of provoking inflammation in animal models are more time-consuming. A recent work, primarily addressing steroid-induced atrophy, used two models to assess the anti-inflammatory effect of two steroids (13). One model, in the hairless mouse, required 5 days' treatment of trunk skin with croton oil, followed by 5 days' treatment with the steroid. The other, evaluating 12–0-tetradecanoyl-phorbol-13-acetate-induced ear edema, required large groups of mice. The trunk skin-croton oil model of inflammation has not been tested for sensitivity to steroid potency. The ear edema model, while widely used, is apparently quite variable over time. It has been suggested that a full time course is necessary for meaningful results (14).

Atrophy in the hairless mouse had been shown to be sensitive to steroid potency (15) but required 18 consecutive days of treatment. Another study, assessing the effect of four steroids applied for 21 days to hairless mice, reported potency differ-

ences in atrophogenesis (16). Unfortunately, opposite flanks of the same animal were used for treatment and control. In such a small thin-skinned animal, not only does transfer of material occur, but topical applications rapidly become systemic, confounding interpretation.

Because of the large numbers of steroids we wished to test, we were unable to commit more than two mice per treatment group. In view of our experience in subsequent studies (17), we would recommend five mice per group to accommodate full statistical analysis. Nevertheless, the results with our model, utilizing small numbers of animals and short treatment periods, show good correlation with the demonstrated efficacy of these proprietary formulations in moderating inflammatory skin disorders and psoriasis (1). Only an occasional disparity with vasoconstriction assays was noted. In our model, for example, halcinonide cream was weaker than in the vasoconstriction data (1). In another case, our model proved more discriminating than the vasoconstriction assay. Stoughton (18) reported no difference between three concentrations (0.025, 0.1%, 0.5%) of fluocinolone acetonide, whereas we found that 0.1 and 0.5% were clearly different.

Experience has shown that the vehicle has a powerful influence on anti-inflammatory potency. The same steroid may be classified into different categories of clinical potency depending on the composition of the vehicle. This model should be useful for optimizing steroid formulations.

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II. Assessment of Name Brand versus Generic Potency

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The hairless mouse model of a UVA-induced dermal neutrophilic infiltrate was used to compare the efficacy of equal concentrations of name brand versus generic corticosteroids. The generic brand was significantly less effective in suppressing the inflammatory response.

Within the last decade there has been over a five-fold increase in the United States in the number of generic products reaching the market (1). With regard to corticosteroids, serious questions have been posed about the equivalence of generic products in comparison to the name brand (1–3). A number of studies have put the question to test using the human vasoconstriction assay (4–6). In two studies (5, 6) the generic betamethasone dipropionate was found to be less potent than the name brand. A similar discrepancy was found with betamethasone valerate preparations (6). Interestingly, Stoughton (4) found no difference between three concentrations (0.025, 0.1, 0.5) of Kenalog® cream (triamcinolone acetonide), but the lowest concentration was still more potent than the generic cream at 0.1%.

After we developed the UVA-neutrophil assay for the anti-inflammatory efficacy of corticosteroids (7), we were interested in using the model to address the generic question. We included, within a series of different potency corticosteroids, two concentrations of desoximetasone (Topicort®, 0.05 and 0.25% emollient cream) and a generic brand (Taro, 0.05 and 0.25%). As in other published reports, we found a significant reduction in potency in the generic product.

MATERIALS AND METHODS

The UVA source, irradiation parameters and quantification of neutrophils are described in the accompanying paper (7). In this study there were some differences in methodology. Briefly, the Skh-hairless-1 (albino) mice were obtained from Charles River Laboratories (Wilmington, MA, USA) and there were 5 animals per treatment group.

To prevent undue discomfort to the mice, irradiation was confined to a 2×2 cm square of the dorsal trunk which was outlined with opaque tape. The remainder of the dorsal surface was treated with a broad spectrum (sun protection factor 15) sunscreen. Because exposure time was 200 min, mice were anesthetized with an intraperitoneal injection of 100 μl of a 1:9 dilution of γ-hydroxybutyric acid lactone (Sigma Chemical Co., St. Louis, MO).

In order to better approximate human use of corticosteroids, treat-

ment schedule in this study was longer than that used in the development of the assay (7). Steroid treatment was once daily for 7 days. Irradiation was on the 8th day. The results of neutrophil quantification were analysed by a paired *t*-test at the 95% confidence level.

RESULTS

All steroid-treated animals had significantly less neutrophil infiltration than the UVA controls (Figs. 1 and 2). Additionally, the higher potency steroids reduced the neutrophil count to a significantly greater degree than those of lower potency (Fig. 1).

A comparison of the higher concentration (0.25%) generic desoximetasone to the name brand (Fig. 2) showed the latter to be significantly more potent in reducing the neutrophil count ($p = 0.004$). The difference between the two brands at 0.05% was not significant, but there was a trend for the name brand to be more potent than the generic. However, the lower concentration name brand steroid (0.05%) was marginally significantly more potent than the high concentration (0.25%) generic ($p < 0.05$). The name brand, at the two concentra-
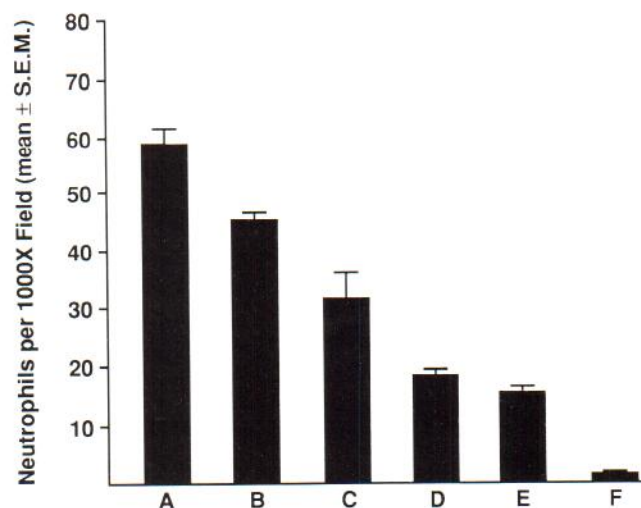

Fig. 1. Comparative potency of various steroids: average number of neutrophils per 1000X field ± S.E.M.

Key *A*: UVA only. *B*. hydrocortisone (Hytone 1% cream). *C*. triamcinolone acetonide (Aristocort A 0.1% cream). *D*. fluocinonide acetonide (Lidex 0.05% cream). *E*. clobetasol-17-propionate (Termovate 0.05% cream). *F*. unirradiated control.

Statistical analysis: paired *t*-test at 95% confidence level.

All steroids significantly different from UVA only: $p < 0.01$ to 0.0001. *B* vs *C*: $p = 0.03$. *B* vs *D*: $p = 0.0003$. *B* vs *E*: $p = 0.0001$. *C* vs *D*: $p = 0.04$. *C* vs *E*: $p = 0.02$. *D* vs *E*: N.S. *E* vs *F*: $p < 0.0001$.