

## The Hairless Mouse as a Model for Study of Local and Systemic Atrophogenic Effects Following Topical Application of Corticosteroids

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The hairless mouse has been used as a model to distinguish between local and systemic atrophogenic effects of topical steroids. Hydrocortisone-17-butyrate, betamethasone-17-valerate, budesonide and clobetasol-17-propionate were applied topically daily for 21 days. Skinfold thickness and dermal DNA synthesis of treated and untreated skin were evaluated as parameters of local and systemic atrophogenicity. Further, body weight gain and thymus weight were assessed as markers of systemic activity. With respect to local effects, skin thickness and dermal DNA synthesis both proved to be good parameters. Of the systemic parameters, thymic involution and body weight gain paralleled quite well the skin thinning on the untreated side. The results confirmed the potency differences of the steroids. Furthermore, they emphasize the usefulness of the hairless mouse to assess the relative safety with respect to local and systemic side effects of chronically applied topical corticosteroids. **Key words:** Systemic activity; Thymic involution; DNA synthesis; Body weight gain; Topical corticosteroids.

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Anti-inflammatory steroids are known to inhibit cell division and to depress protein and mucopolysaccharide synthesis. These effects have been claimed, to explain – at least partly – epidermal thinning and dermal atrophy. The latter are well known side effects following the use of topical corticosteroids (1). Considerable effort has been expended in searching for compounds with potent anti-inflammatory activity, but with little propensity to cause skin atrophy. However, these attempts have been unsuccessful as yet, since the atrophogenic activity was found to correlate strongly with the intrinsic potency of the topical steroid.

In the present study we evaluated the local and systemic atrophogenic activity of some corticosteroids by using the hairless mouse model.

### MATERIALS AND METHODS

#### Animals

Male hairless mice (strain hr/hr-NMRI) weighing  $20 \pm 2$  g were used. The animals were randomly divided into five groups of 7. On the left flank, a skin area of  $1 \times 1.5$  cm was marked and 10  $\mu$ l of one of the test formulations was applied once daily (in the morning) for 3 weeks. The right flank was left untreated.

#### Skinfold thickness/body weight

On day 0, 3, 7, 10, 14 and 21, body weight was measured and skinfold thickness of treated and untreated sides was determined, using a graduated micrometer (Mitutoya). On day 21 the animals were sacrificed and dermal thymidine uptake and wet weights of thymus were determined.

#### Thymidine uptake

Dermal DNA synthesis of treated and untreated skin was determined by the disc technique of Otani et al. (2).

#### Formulations

(i) Untreated (control); (ii) hydrocortisone-17-butyrate (HCB) 0.1% (Locoid lipocream: Gist-brocades); (iii) betamethasone-17-valerate (BV) 0.1% (Betnelan-V ointment: Glaxo); (iv) budesonide (Bud) 0.025% (lipocream: Gist-brocades); (v) clobetasol-17-propionate (CP) 0.05% (Dermovate ointment: Glaxo).

#### Statistical analysis

Mean values of the different parameters for each treatment group were statistically compared with corresponding untreated control values using Dunnett's *t*-test at the significance level of  $p < 0.05$ . As BV/HCB and CP/Bud are considered to belong to two different classes of corticosteroid potency, the effects were further compared within each potency group. Systemic and local corticosteroid activities were checked for correlation, using the Spearman correlation.

### RESULTS

The results are presented in Figs. 1–3. The various systemic parameters in this study were checked for coherence by determining the Spearman correlation coefficient for datasets obtained from the individual animals. Positive correlations were found for the gain in body weight versus the thymus weight at day

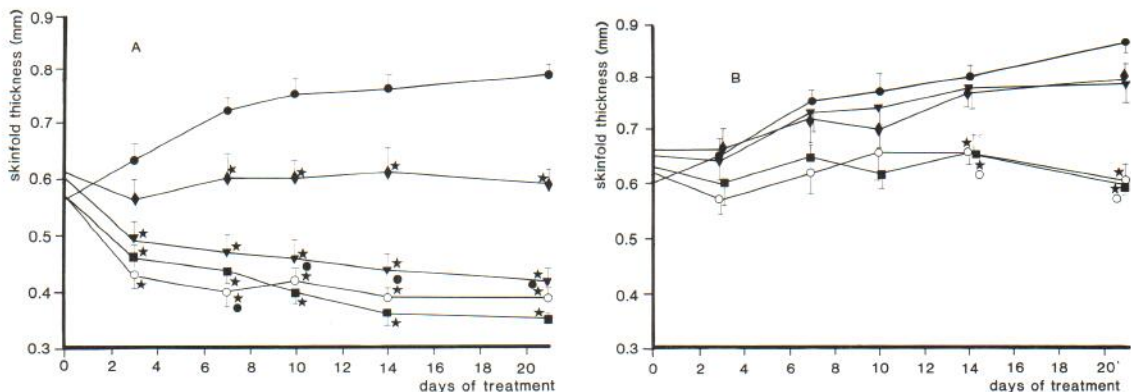


Fig. 1. Skinfold thickness (mean  $\pm$  SE) at different application times of treated (A) and untreated sides (B) following no treatment (●—●, control) and daily topical application of HCB (◆—◆), BV (○—○), Bud (▼—▼) and CP (■—■). Symbols indicate significant differences as compared with corresponding control (★), HCB (●) and Bud (○) values respectively (Dunnett's *t*-test,  $p < 0.05$ ).

21 of study (0.741); for the skinfold thickness on the right side versus the thymus weight at day 21 (0.569), and for the skinfold thickness on the right side versus the dermal thymidine uptake of the right side at day 21 (0.538). All other correlations of the systemic parameters showed a correlation coefficient lower than 0.5.

With respect to the parameters of local effect of the corticosteroids, a positive correlation (0.669) was found for the decrease in skinfold thickness versus the dermal thymidine uptake on the left side (both at day 21). The relative local versus systemic effect of the drugs was calculated using the mean skin thickness data (ST) for each group of animals on the treated ( $l = \text{left}$ ) and untreated ( $r = \text{right}$ ) side (Cont. = control group) in the following equation (Table I):

$$\begin{aligned} \text{Index of local versus systemic effect} &= \\ &= \frac{(ST_l X - ST_r \text{ Cont.})/ST_r \text{ Cont.}}{(ST_l X - ST_l \text{ Cont.})/ST_l \text{ Cont.}} \end{aligned}$$

With respect to this index the ranking of the preparations was: Bud < HCB < CP < BV.

## DISCUSSION

In clinical practice, cutaneous atrophogenic effects of topical corticosteroids have been looked upon as side effects, although it cannot be denied that in the treatment of psoriasis the antimetabolic properties of these compounds are essential. Therefore, in spite of all efforts to distinguish between dermal or epidermal atrophy parameters and clinical effectiveness or between dermal and epidermal glucocorticosteroid receptors, one must accept the pharmacological indi-

Table I. Local versus systemic effects of corticosteroid preparations with skinfold thickness (left versus right) as parameter

Treatment group	Skin thickness <sup>a</sup> left ( $\pm$ SE)	% <sup>b</sup>	Skin thickness <sup>a</sup> right ( $\pm$ SE)	% <sup>b</sup>	Index right/left
Control	0.79 $\pm$ 0.046	—	0.87 $\pm$ 0.050	—	—
HCB	0.59 $\pm$ 0.068	25	0.80 $\pm$ 0.080	8	0.32
Bud	0.42 $\pm$ 0.062	47	0.79 $\pm$ 0.087	9	0.19
BV	0.40 $\pm$ 0.058	49	0.62 $\pm$ 0.077	29	0.59
CP	0.35 $\pm$ 0.026	56	0.61 $\pm$ 0.049	30	0.54

In each group, 7 animals were tested, except for the control group (6).

<sup>a</sup>Mean values (mm).

<sup>b</sup>Thickness of (treated - control)/control  $\times$  100% for left or right side, respectively.



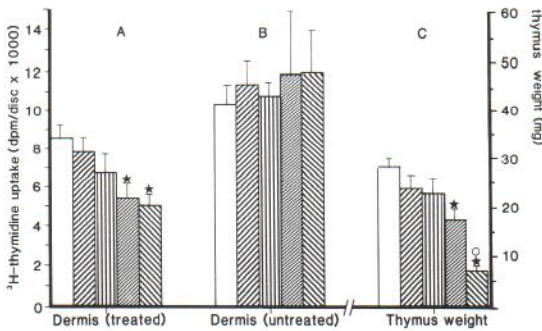


Fig. 2. Dermal tritiated thymidine-uptake of treated (A) and untreated sides (B), wet thymus weight (C) at day 21 following no treatment ( $\square$ , control) and daily topical application of HCB ( $\square$ ), BV ( $\blacksquare$ ), Bud ( $\square$ ) and CP ( $\square$ ) (mean  $\pm$  SE). Symbols indicate significant differences as compared with corresponding control ( $\star$ ) and Bud values ( $\circ$ ) respectively (Dunnett's *t*-test,  $p < 0.05$ ).

stinctiveness of the receptors involved in both atrophy and anti-inflammatory potential of these drugs.

In the present study, [ $^3$ H]thymidine incorporation was taken as a parameter for both local and systemic effects of the drugs. As could be expected from the data of Du Vivier (3), but in contrast to the results of Lubach & Kietzmann (4), inhibition of epidermal [ $^3$ H]thymidine incorporation faded readily (authors, data not shown). Therefore, it proved useful to separate dermis and epidermis in this assay, since in the dermis a strong anti-mitotic effect could be shown.

Of the parameters used in this study to assess the systemic effects of the corticosteroid preparations, the gain in body weight of the (young) animals was shown to be a more sensitive index of systemic effect than was the skin-thickness or [ $^3$ H]thymidine incorporation on the contralateral (untreated) flank side, and paralleled the thymic involution data.

With respect to local effects, skin-thickness was a very sensitive parameter. Inhibition of the incorporation of [ $^3$ H]thymidine was found to be a good parameter too. Moreover, the correlation between the two parameters was acceptable. The present data showing a lower systemic index of HCB vis-à-vis BV is consistent with clinical data (5) where BV and HCB showed equal potency but a weaker effect of the latter on the HPA axis. For the new steroid, budesonide, this study confirms the findings of Gruvstad & Bengtsson (6) who demonstrated the potent corticosteroid effects of budesonide in the vasoconstrictor assay. The relatively small effect of budesonide on systemic parameters in this study, as

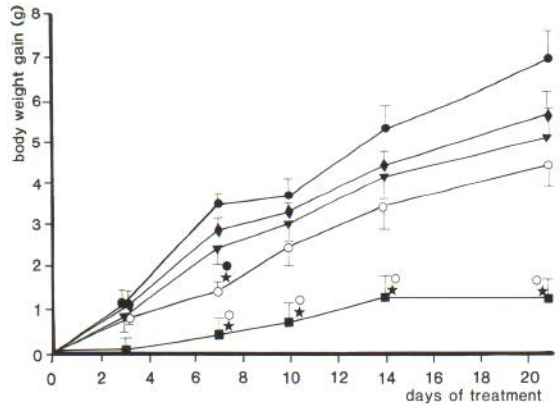


Fig. 3. Body weight gain (mean  $\pm$  SE) at different intervals following no treatment ( $\bullet$ — $\bullet$ , control) and daily topical application of HCB ( $\blacklozenge$ — $\blacklozenge$ ), BV ( $\circ$ — $\circ$ ), Bud ( $\blacktriangledown$ — $\blacktriangledown$ ) and CP ( $\blacksquare$ — $\blacksquare$ ). Symbols indicate significant differences as compared with corresponding control ( $\star$ ), HCB ( $\bullet$ ) and Bud ( $\circ$ ) values respectively (Dunnett's *t*-test,  $p < 0.05$ ).

indicated by its low index of systemic effect, is in line with the results of Salde (7) who showed a relatively low systemic effect combined with a strong local effect of topical budesonide in patients. It seems very likely that the weak systemic effect of this drug is due to the rapid decline in plasma levels following systemic absorption of the drug (8).

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