Topical Application of Clobetasol-17-propionate Inhibits the Intra-epidermal Accumulation of Polymorphonuclear Leukocytes

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The in vivo effect of topical clobetasol-17-propionate (CP) on the leukotriene B_4- and trauma-induced intra-epidermal accumulation of polymorphonuclear leukocytes (PMN) was investigated in normal volunteers. PMN accumulation was assessed using elastase, a specific and sensitive marker for these cells. A profound inhibition of PMN accumulation was shown in the first 2 days of treatment with CP, an effect that faded away after a treatment of 2 weeks.
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Clobetasol-17-propionate (CP) is a potent corticosteroid preparation with an exceptional clearing capacity in psoriasis (1). A wide range of working mechanisms have been reported, including an antimitotic effect, normalization of keratinization pattern and immunomodulations (2).

The intra-epidermal penetration of polymorphonuclear leukocytes (PMN) is an early event in the pathogenesis of the psoriatic lesion and their arrangement as spongiform pustules is characteristic of the disease (3).

In the present study, normal skin was treated with CP creme and its vehicle only (placebo), and following a chemotactic signal, the intra-epidermal accumulation of PMN was measured. The enzyme elastase which allows accurate quantification of skin PMN was used as marker (4). The chemotactic signals were:

(i) topical application of leukotriene B_4 (LTB_4), which produces microabscesses of PMN in the epidermis (5, 6).
(ii) standardized injury (sellotape stripping), which brings about an endogenous release of chemoattractants and causes intra-epidermal accumulation of small amounts of PMN (7).

MATERIALS AND METHODS

Subjects and treatments

Eight subjects without signs or previous history of skin diseases participated in this study. The group consisted of 5 females and 3 males, aged between 22 and 31 years. Prior consent of the Medical Ethics Committee was obtained for these experiments.

In a double-blind approach, 0.05% CP creme (Dermovate Creme®, Glaxo) and the placebo (skin base creme®, Glaxo) were applied thinly and evenly without occlusion on two different demarcated test areas (10×10 cm) localized on the left or right upper arm. The cremes were applied twice daily for a period of 2 weeks, except on the day of LTB_4 application and sellotape stripping.

LTB_4 application and sellotape stripping

LTB_4 was purchased from Paesel GmbH Frankfurt, Germany. Twelve hours after the second application of CP and placebo creme the test areas were cleansed with 70% ethanol. Aliquots of 20 ng LTB_4 in ethanol were then applied onto 5.5 mm circular patches within the CP and placebo test areas, as well as on an untreated skin region of the upper arm. The ethanol was evaporated with a stream of nitrogen. The three LTB_4 application sites were marked with eosin and covered with impermeable dressings (Silverpatch, van der Bend, Brielle, The Netherlands). Biopsies of LTB_4 treated sites were taken freehand using a razor-blade in conjunction with a metal guard 24 h following the application (time of maximum accumulation of PMN). Treatment with CP creme and placebo creme was continued for 14 days. Twelve hours after the last application of these cremes, the procedure of LTB_4 application followed by biopsy was repeated for the CP- and placebo-treated areas.

Twelve hours after the second application of the cremes, three circular regions (15 mm ∅) were stripped by repeated application of sellotape. Two of the regions were within the CP- and placebo-treated areas; the third was an untreated area on the upper arm. Removal of the stratum corneum was considered to be complete when the whole area appeared to be glistening (30–40 applications). Razor-blade biopsies in combination with a metal guard (hole 5.5 mm ∅) were taken from the centre of the stripped sites 8 h later (time of maximum accumulation of PMN). After 14 days of treatment (12 h after the last application of the cremes) this procedure was repeated for the CP- and placebo-treated areas.

Analytical procedures

Biopsies (average fresh weight 3 mg) were processed for elastase measurement as described previously (4). In brief, biopsies were homogenized in cetrimide buffer after thorough washing in phosphate-buffered saline. Elastase activity was measured in duplicate 20-µl aliquots of the supernatants from the fluorescence of 7-amino-4-methylcoumarin released from the substrate MeOSuc-Ala-Ala-Pro-Val-N-methylcoumarin. The density of infiltrating PMN per 10 µg skin was calculated after correction for elastase inhibitors (4).

Statistical analysis

The Wilcoxon ranking test for paired data was used in the statistical analysis. Level of significance, 0.05.

RESULTS

During the first days of treatment, no blanching occurred. After about one week the CP-treated areas of all subjects showed mild erythema with slight scaling, whereas the placebo-treated area showed no abnormalities. In 2 subjects the picture was complicated by folliculitis within the CP-treated test areas.

PMN accumulations 24 h following LTB_4 applications are given in Fig. 1. After two applications of CP, a reduced responsiveness occurred vis-à-vis the placebo-treated area or untreated area (both, $p<0.025$). After 14 days of treatment with CP, no significant differences could be demonstrated between the CP-treated, placebo-treated and untreated areas.

PMN accumulations 8 h following sellotape stripping are given in Fig. 2. After two applications of CP a markedly reduced responsiveness occurred, vis-à-vis the placebo-treated and untreated areas ($p=0.01$ and $p<0.01$, respectively). After 14 days of treatment with CP, no significant difference could be shown vs. placebo-treated or untreated areas.

DISCUSSION

After CP treatment for one day, LTB_4-induced PMN accumulations were reduced to 30% of the responses observed following treatment with the placebo. Trauma-induced PMN accumulations were inhibited even more profoundly; in the CP-treated areas they averaged only 12% of the responses measured after treatment with the vehicle. After a treatment period of 14 days the situation was less certain; PMN accumulation could not be shown to be significantly affected by CP treatment. However, all subjects developed a slight dermatitis and a folliculitis was seen in 2 of them, which could account for the wide range of PMN accumu-

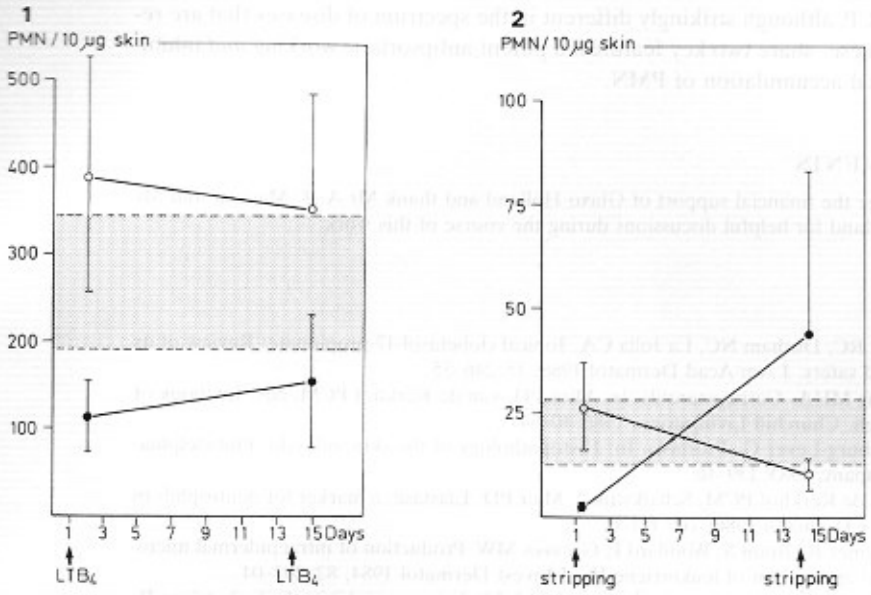

Fig. 1. Intra-epidermal accumulation of polymorphonuclear leukocytes 24 h after topical application of 20 ng leukotriene B_4 on the skin of 8 normal volunteers. The shaded area represents the normal range (mean ± SE) of untreated skin following LTB_4 application. ○, Skin treated with placebo creme (mean ± SE); ●, skin treated with clobetasol-17 propionate creme (mean ± SE).

Fig. 2. Intra-epidermal accumulation of polymorphonuclear leukocytes 8 h following sellotape stripping of the skin of 8 normal volunteers. The shaded area represents the normal range (mean ± SE) of untreated skin following sellotape stripping. ○, Skin treated with placebo creme (mean ± SE); ●, skin treated with clobetasol-17 propionate creme (mean ± SE).

lations. Such clinical effects have been observed occasionally in patients treated with CP creme twice daily (1).

The present in vivo study, with the dermis and epidermis acting as a natural diffusion chamber, demonstrates that the LTB_4-induced chemotaxis is reduced following CP treatment. A more precise localization of the action site of CP would require in vitro studies. The PMN itself could well be the target cell for corticosteroids. Indeed it has been shown that incubation of PMN derived from peripheral blood with corticosteroids reduces the chemotactic activity of these cells (8). An alternative explanation for the action of CP might be an interference with the interaction between PMN and endothelial cells. Several authors have reported the aggregation of PMN and the adhesion of these cells to the endothelium induced by mediators of inflammation including LTB_4 (9, 10).

It has been shown that corticosteroids inhibit the adhesion of PMN to endothelial cells in vitro (11), which might reduce PMN passage into the skin.

Following trauma, mediators of inflammation are released, including arachidonic acid metabolites. Corticosteroids have been reported to inhibit arachidonic acid release by inducing the phospholipase A_2 inhibitor lipomodulin (12). Thus, in trauma-induced PMN accumulation a synergistic modulation by CP is feasible: inhibition of endogenous release of mediators of inflammation and inhibition of the effect of the mediator LTB_4 on PMN penetration.

In previous studies we have shown that systemic treatment with etretinate and methotrexate in dosages used for the treatment of psoriasis inhibits the LTB_4-induced intra-epidermal accumulation of PMN (6, 13, 14). It is of interest that these systemic treatments and

topical therapy with CP, although strikingly different in the spectrum of diseases that are responsive to each of these, share two key features: a potent antipsoriatic working *and* inhibition of intra-epidermal accumulation of PMN.

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