

Prolonged treatment with topical glucocorticoids results in an inhibition of the allergen-induced weal-and-flare response and a reduction in skin mast cell numbers and histamine content

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Summary

The inhibitory effects of topical glucocorticosteroid treatment on the immediate dermal allergic reaction were studied in 10 patients in a double, randomized, placebo-controlled fashion. The aim was to study whether a prolongation of the treatment time would enhance the inhibitory effect beyond the 30–40% reduction previously reported after 1 week of treatment, and whether any changes in skin reactivity were accompanied by changes in the level of mast cells or histamine at the challenge site. Allergen and histamine skin-prick tests were performed on both forearms before the start of the study and after 2 and 4 weeks of treatment with placebo cream on one forearm and with 0.05% clobetasol-17-propionate cream on the other. Punch biopsies from the skin treated actively and with placebo were taken after 4 weeks in eight of the patients. The specimens were used for the light-microscopic evaluation of mast cell density and for the measurement of histamine and protein content. After 4 weeks of treatment we found a reduction in the allergen-induced weal (72%; $P < 0.001$) and flare (62%; $P < 0.05$) response. There was also a minor reduction in the histamine-induced weal (38%; $P < 0.05$) but not the flare response, suggesting that the glucocorticoid treatment induced a reduced mediator release at allergen challenge. This could be partially explained by the finding of a reduction in the number of detectable skin mast cells (85%; from 0.78 to 0.11 mast cells per unit area) and in the histamine content of the skin as related to the tissue wet weight (36%; $P < 0.05$). There was also an increase in the protein content of the skin in relation to tissue weight (37%; $P < 0.05$), which was presumably partly caused by a reduction in the water content of the skin, further enhancing the reduction of histamine when related to tissue protein content to 56% ($P < 0.01$). These findings of a glucocorticosteroid induced reduction in the mast cell density of the skin differ from the results of our previous study of airway mucosa, where the mast cell density was not reduced by topical glucocorticoid treatment in a dosage which effectively inhibits the immediate allergic response after allergen challenge. These differences can be interpreted as being due to differences in the steroid responsiveness of different mast cell populations, but may also depend on differences in the pharmacokinetics of the glucocorticosteroids when applied locally to the skin as opposed to the airway mucosa. The present results emphasize the role of the mast cell and histamine in the pathogenesis of the immediate dermal allergic reaction, but also stress that the effects of glucocorticosteroids are largely dependent not only on dosage but also on treatment times. Results from *in-vitro* and other studies involving short-term treatment with glucocorticosteroids should therefore be interpreted with caution.

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Introduction

A variety of dermal diseases are treated with topical glucocorticosteroids, and the dermal use constitutes the current major clinical application of these hormones. Although clinically effective, their precise mode of action is still largely unclear [1]. Glucocorticosteroids were previously believed to have no effect on the immediate allergic reaction as induced by local allergen challenge in the upper and lower airways [2,3] in contrast to the late phase response and allergen-induced hyperreactivity in the lower airways which are prevented by glucocorticoid treatment [4]. It is becoming increasingly clear, however, that the glucocorticosteroids can actually augment the initial part of the allergic response to allergen. This has been shown most convincingly in nasal challenge experiments [5–7], but there are also similar reports from bronchial [8] and dermal experiments [9]. It appears that the prerequisite for the demonstration of any effect on the immediate allergic reaction in the nasal mucosa is that the glucocorticosteroids are applied topically for a prolonged time, for at least 36–48 hr, depending on which parameter is being measured [10]. Similarly, 1–2 weeks of topical dermal glucocorticoid treatment was reported as resulting in a 30–40% inhibition of the immediate response to allergen challenge in addition to a more or less complete obliteration of the late phase cutaneous response [9]. Whether the reaction can be inhibited even further by longer treatment times is at present unclear, as is the cellular basis for the inhibitory effect of the glucocorticosteroids. However, very prolonged treatment with corticosteroids may reduce both mast cell numbers and histamine content in the human skin. Thus the local application of two potent corticosteroids, clobetasol-17-propionate and fluocinolone, produced a decrease of >85% in histamine content over a 6-week treatment period, whereas the electron-microscopic examination of the biopsies indicated that the reduced levels of histamine were caused by a depletion of mast cells [11].

The aim of the present investigation was to explore the time-dependence of the action of topical dermal glucocorticosteroid treatment on the immediate allergic response and to investigate whether changes in skin reactivity were accompanied by any changes in mast cell numbers and histamine content of the tissue.

Materials and methods

Study design

The study was designed as a double-blind, randomized within-patient study with active treatment on one forearm and placebo treatment on the other forearm for 4 weeks. Skin-prick tests were performed on the treated

areas of both forearms immediately before the start of the study and after 2 and 4 weeks of treatment. Skin-punch biopsies were performed after 4 weeks on the treated areas of the skin. The specimens were used for light-microscopic analysis, including the determination of mast cell density, and for the biochemical assay of protein and histamine content.

Patients

Ten male patients aged 19–31 years (mean age 21.1 years) were recruited for the study. They all had a history of allergic rhinitis, verified by positive skin-prick tests for birch pollens (*Betula verrucosa*) and/or timothy pollens (*Phleum pratense*), and did not show any bronchial or dermal manifestation of their allergic disease. No medication was allowed for at least 3 weeks before entering into and during the trial, which took place during the pollen-free autumn and winter months. The investigation was performed according to the rules laid down in the Declaration of Helsinki and was approved by the Ethics Committee of the University of Lund.

Test procedure

After an initial skin-prick test the patients entered the double-blind part of the study. They received two tubes of dermal preparation, one marked left arm and the other marked right arm. One of these tubes contained a cream preparation of 0.05% clobetasol-17-propionate (DermovateTM; Glaxo Pharmaceuticals, Gothenburg, Sweden) and the other contained the corresponding vehicle. The preparations were identical in appearance, and neither the patients nor the investigator knew which preparation contained the active compound. The patients were instructed to apply the topical preparations as a thin film on a marked area (~20 × 10 cm) on the volar aspects of both forearms in the morning and evening for 4 weeks. The skin-prick test was repeated on both arms in the pretreated skin areas after 2 and 4 weeks of treatment, 6–8 hr after the latest application of cream.

Prick tests

At an initial visit a skin-prick test was performed on the volar aspect of the forearm with histamine and either birch pollen (three patients) or timothy pollen extract (seven patients). Preloaded standardized skin-prick devices (PhazelTM; Pharmacia AB, Uppsala, Sweden) were used [12]. The weal-and-flare responses obtained after 15 min were outlined with a felt pen and transferred onto paper with the help of an adhesive plastic tape. The area was then measured using a digitizer (MacintizerTM,

GTCO, Columbia, MD, U.S.A.) served by a microcomputer (Apple Macintosh™, Cupertino, CA, U.S.A.).

Morphological techniques

Two punch biopsies (2 mm in diameter) were obtained from each forearm at the final, 4-week follow-up visit from eight of the patients. The biopsy specimens were taken after local infiltration anaesthesia (Mepivacaine 5 mg/ml + adrenalin 5 µg/ml; Carbocain®-adrenalin; Astra, Södertälje, Sweden) on the previously treated skin in an area not used for the prick tests. One of the biopsy specimens from each forearm was weighed and then immediately frozen and stored at -70°C for subsequent biochemical analysis. The other biopsy, intended for light microscopy, was treated as previously described [13,14]. After sectioning, the specimens were stained with toluidine blue for the specific visualization of mast cells (0.5% toluidine blue in 0.5 M HCl for 30 min). All the mast cells in two sections from each biopsy were counted. The area of the section was determined by means of planimetry using a digitizer served by a microcomputer (as detailed above) and the number of mast cells was expressed per unit area of tissue.

Biochemical analyses

The biopsy specimens weighing 1.5–6.8 mg were stored at -70°C and homogenized in 0.4 M perchloric acid followed by the neutralization of the extract and the precipitation of potassium chlorate. Histamine was purified by ion pair chromatography on an C18-column in a reverse-phase HPLC system using a 15 mmol citrated buffer at pH 3 containing 5% methanol as the mobile phase and pentane sulphonic acid (5 mmol) as the counter ion. Histamine was detected and assayed using a two stage post-column derivative forming procedure using *o*-phthalaldehyde as the fluorescent reagent. Details of the procedure are published elsewhere [15]. The protein content of the biopsy specimen was also determined according to Lowry *et al.* [16] and the histamine content was also expressed as histamine/protein quotients.

Statistics

Areas of weal-and-flare obtained at the three skin-prick tests on each forearm initially and after 2 and 4 weeks of treatment were analysed using an analysis of variance for repeated measurements. When a statistically significant difference was obtained a further analysis was performed using a Student's *t*-test for paired observations. The same test was used for the analysis of paired obtained from the individual treated actively and with placebo. An analysis

of the correlations between the dermal histamine content, mast cell density and skin-prick tests was also made. The calculations were performed on a microcomputer with the aid of a statistical software package.

Results

Skin-prick test

The results of the skin-prick tests are available from all 10 individuals. The allergen-induced weal-and-flare reactions are presented in Fig. 1, and the corresponding histamine-induced reactions in Fig. 2. As shown, there was a drastic reduction in the allergen induced weal-and-flare response with a statistically significant difference between the arms treated actively and with placebo after 2 ($P < 0.05$ weal; $P < 0.05$ flare) as well as 4 ($P < 0.001$ weal; $P < 0.05$ flare) weeks. A considerably smaller, but statistically significant difference was noted for the histamine-induced weal after 2 ($P < 0.05$) as well as after 4 ($P < 0.05$) weeks, while no influence was observed on the histamine-induced flare.

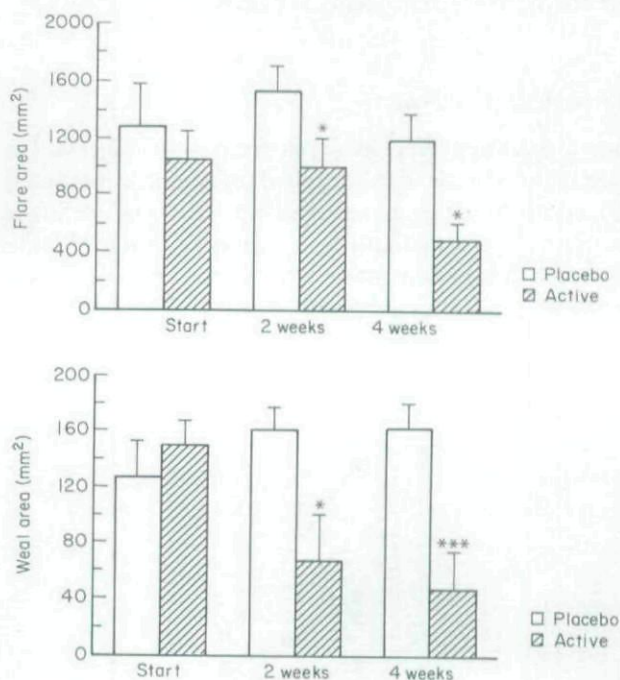


Fig. 1. Area of allergen-induced weal (upper panel) and flare (lower panel) reactions before and after 2 and 4 weeks of topical dermal treatment with 0.05% clobetasol-17-propionate. (□) Placebo-treated skin; (▨) actively treated skin. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (active *vs* placebo).

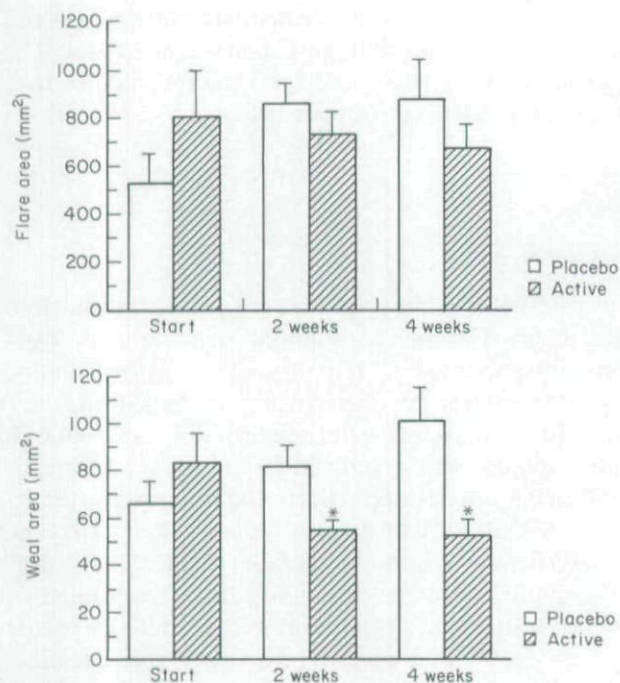


Fig. 2. Area of allergen-induced weal (upper panel) and flare (lower panel) reactions before and after 2 and 4 weeks of topical dermal treatment with 0.05% clobetasol-17-propionate. (□) Placebo-treated skin; (▨) actively treated skin. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (active vs placebo).

Morphological findings

Biopsy specimens were available from eight subjects. The staining technique used resulted in metachromatically stained mast cell granules against a virtually unstained background thus facilitating the counting procedure. The placebo-treated skin specimens contained a variable number of mast cells generally located in the subepithelial

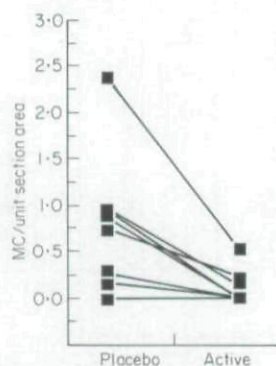


Fig. 3. Individual difference in mast cell number in skin biopsies taken in placebo-treated and actively treated skin. The difference is statistically significant $P < 0.01$ (active vs placebo).

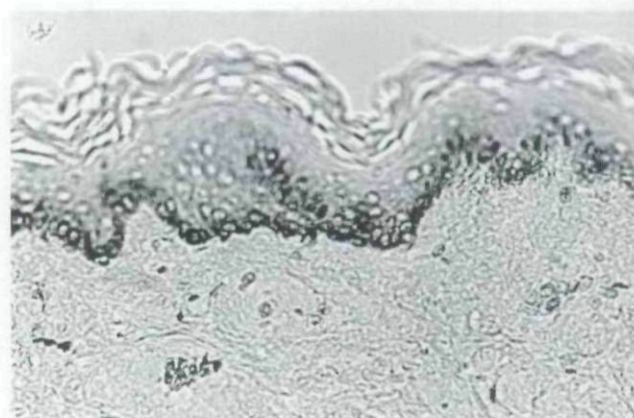


Fig. 4. Section from a skin biopsy taken in placebo-treated skin. A toluidine stained section with a weak background stain and a metachromatic staining of mast cell granules is shown ($\times 230$).

dermal area and around the dermal blood vessels. The actively treated skin specimens were notably depleted of stainable mast cells. The individual differences in the number of mast cells are presented in Fig. 3. The mean number was reduced from 0.78 ± 0.26 to 0.11 ± 0.07 mast cells/unit section area ($P < 0.01$). An example from one patient is demonstrated in Fig. 4 showing a skin biopsy section of a placebo-treated skin specimen using a staining technique which facilitates the mast cell counting process. The actively treated skin specimen appeared to exhibit some degree of skin atrophy, as indicated by an apparent reduction in the epithelial thickness, but no efforts were made to quantify such changes further. There was a statistically significant correlation between the number of mast cells in the skin of the untreated skin area and the size of the allergen-induced weal both for the

Table 1. Content of histamine and protein in the skin after 4 weeks of topical treatment with 0.05% clobetasol-17-propionate

Component	Placebo treatment (mean $\pm$ s.e.m., $n = 8$)	Active treatment (mean $\pm$ s.e.m., $n = 8$)
Histamine/tissue wet weight ($\mu\text{g/g}$)	4.09 ± 0.35	$2.61 \pm 0.49^*$
Protein/tissue wet weight (mg/g)	131 ± 16	$185 \pm 15^*$
Histamine/protein quotient (ng/mg)	34 ± 5	$15 \pm 3^\dagger$

* $P < 0.05$.

† $P < 0.01$.

placebo-treated ($r=0.72$; d.f. = 7; $P<0.05$) and the actively treated skin ($r=0.84$; d.f. = 7; $P<0.01$).

Biochemical findings

The mean ($\pm$ s.e.m.) histamine and protein content of the skin specimens is presented in Table 1. The active treatment induced a 36% reduction ($P<0.05$) in the levels of histamine in relation to tissue weight ($P<0.05$). The glucocorticosteroid treatment also resulted in a 37% increase in the protein content of the dermal specimens in relation to wet weight ($P<0.05$). The histamine/protein quotient was thus reduced by 56% ($P<0.01$). In the untreated specimens the correlation coefficient between the mast cell density and histamine/protein content was $r=0.47$ (n.s.) and $r=0.53$ (n.s.) for the treated specimens.

Discussion

It has been suggested that the size of the allergen-induced weal-and-flare reaction reflects the density of IgE on the mast cells and thereby also the severity of the allergic disease [17–19]. From the present study it appears that the size of the skin reaction also depends, at least to some extent, on the density of mast cells in the skin.

In a previous study, where a similar design and drug was used, treatment for 1 week induced a 35% reduction in the allergen-induced weal and flare response in addition to a more or less total obliteration of a late-phase reaction [9]. By extending the time of treatment a more pronounced reduction in the immediate reaction was obtained in the present study. This points to the treatment time as being a crucial factor for the expression of some glucocorticoid effects. The differences in the steroid responsiveness of the immediate and late phase response in both the airways [7–10,20] and the skin clearly point to differences in the pathogenesis of the two phases of the allergic reaction. The expression of allergic late-phases and allergen-induced hyperreactivity appears to involve a recruitment of circulating granulocytes [20], a process which appears to be highly susceptible to topical glucocorticoid treatment [20] as well as to single intravenous injections of steroids [21]. On the other hand, the expression of an immediate phase appears to be independent of such cells and is probably a direct effect of mediators released from the mast cells.

The effect of the glucocorticoid on the immediate phase response could be the result of a reduction in the density of mediator cells in the skin and/or a reduction in allergen-induced mediator release. The simultaneous reduction of histamine, mast cells, and allergen-induced reactions in the present study provides indirect evidence that at least part of the reduction in responsiveness is due

to a reduction in mediator cell density. This does not exclude that other glucocorticoid effects, such as a direct effect on capillary/blood vessel permeability contribute to the reduction in the allergen-induced reactions. Such a mode of action may explain the reduction in the histamine-induced weal reaction noted in the present study.

The mechanism of the glucocorticoid-induced reduction in mast cell density, which confirms the observations in a previous study by Lavker and Schechter [11], cannot be decided on the basis of the available data. The steroid could act directly on the pre-existing mast cells or block the formation of new mast cells in the skin. The long treatment times required for a reduction in mast cell density would perhaps favour an influence on the mast cell turnover as an explanation for the present findings of the reduction of mast cells. Since the mast cells are identified through a staining of metachromatic granules, a reduction in mast cell numbers could be the result of a degranulation effected by extensive secretory activity, without a corresponding formation of new granules. Since we do not know at what stage of this process the stainability is lost as compared to the capacity to detect histamine in the tissue this may well explain the apparent discrepancy noted in the rate of reduction in mast cell density as compared to the rate of reduction of histamine tissue levels noted in the present study.

Interestingly, this type of glucocorticosteroid-induced reduction in baseline mediator cell density was not found in airway mucosa [13,22,23]. This difference between airway and dermal mast cells might be due to a difference in steroid responsiveness, like the differences in responsiveness observed in dermal and airway fibroblasts [24]. It appears, however, more likely that the difference is due to the major differences in the pharmacokinetics of the glucocorticosteroid. Thus, when applied dermally, glucocorticosteroids are present in the tissue for 24–48 hr after a single application [25], but when given topically on airway mucosa they have been cleared from the mucosa within 1–2 hr [26]. This difference in pharmacokinetics has also been one of the explanations given for the pronounced difference between the airways and the skin when it comes to the induction of glucocorticosteroid induced atrophy [27]. Dermal atrophy is evident after 3 weeks of treatment with the present glucocorticosteroid [28,29], while even year-long treatment of the airway mucosa has failed to produce similar signs of atrophy [30,31].

In conclusion we provide indirect evidence of the participation of the mast cells and histamine in the immediate allergic response. We also demonstrate that glucocorticosteroids, when applied topically for a prolonged time, have a profound inhibitory effect on the immediate allergen-induced weal-and-flare response and

that this might partially be explained by a reduction in the density of skin mast cells.

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