

Effect of Topical Glucocorticoid Treatment on Nasal Mucosal Mast Cells in Allergic Rhinitis

ULF PIKORIN

ENT Department, Dept. of Pathology II and Section of Allergy Medical Dept. I, Sahlgrens Hospital, University of Gothenburg, Sweden

Glucocorticoids were previously considered not to affect the immediate allergic reaction. However, in a nasal allergen challenge, an inhibitory effect on the nasal symptoms induced at the challenge has been shown to occur in patients treated with a recently developed glucocorticoid, budesonide, for 1 week prior to the challenge. This treatment was also found to reduce tissue histamine levels in the nasal mucosa. Mast cells in the mucosa were therefore studied with a view to finding out whether this reduction could be due to a reduction of mast cells. A double-blind study was performed in 14 asymptomatic patients. Nasal biopsies were made before and after 1 week's treatment with either budesonide or placebo. The number of mast cells was counted in two Epon sections after the specimens had undergone specific staining with toluidine blue. No quantitative or qualitative morphological changes in the mast cells were found as a result of treatment.

Key words: allergic rhinitis; glucocorticoid effect; hay fever; mast cells; nasal mucosa.

Accepted for publication 29 October 1982

Although glucocorticoids have gained widespread use in the treatment of allergic airway diseases, the mode of action of the glucocorticoids is not fully understood (6, 18). Topical glucocorticoids with minimal systemic activity have been shown to provide effective and safe treatment in allergic and vasomotor rhinitis (6, 7).

Recently, it has been shown that topical glucocorticoids reduce the immediate allergic reaction to nasal allergen challenge when the pretreatment has been extended to 1 week or longer (10, 12, 20). Possible modes of action on the part of the glucocorticoids have been studied in asymptomatic hay fever patients (13, 14). The glucocorticoid effect on histamine-induced nasal symptoms was found to be minimal (13). In a subsequent study, an *in vitro* challenge with anti-IgE was performed with nasal mucosal specimens from hay fever patients (14). Glucocorticoid pretreatment was found to induce a reduction of histamine content in the mucosa, as well as a marked reduction of the histamine

release induced by anti-IgE. Whether the reduction of the histamine content in the mucosa was caused by a reduction of the number of mast cells, could not be established in that study.

AIM OF INVESTIGATION

The objective of this study was to find out whether topical glucocorticoid treatment with budesonide induced any alteration in the number or structure of nasal mucosal mast cells in asymptomatic hay fever patients.

MATERIAL AND METHODS

Experimental design

The study was designed as a double-blind randomized study with nasal biopsies before and after 1 week of treatment with either budesonide or placebo.

Patients

Fourteen patients (4 females and 10 males) with age ranging from 20 to 34 years (mean age 28.2 years) were selected. All had a typical history of seasonal allergic rhinitis and were skin-prick positive to either birch or grass pollen extracts. Symptoms had been present for the last three seasons at least. They were asymptomatic at the time of the trial, and no other drug was taken during the trial. They were fully informed about the objective and means of the trial and gave their consent to participate in the study. The study was approved by the Ethical Committee of the University of Gothenburg.

The trial was done out of season, during February and March. Biopsy specimens were taken with a small pair of forceps after local anesthesia with a cotton sponge soaked in tetracain. The specimens were taken from the lower edge of the right inferior turbinate about 1 cm behind the front edge. After the first biopsy, the patients received – randomly and blindly – either budesonide in a dose of 200 b.i.d. or placebo in identical freon-propelled canisters. The last dose was given 2 h prior to the second biopsy, which was taken in an identical manner from the left side. Both biopsy specimens were handled identically.

Budesonide

The topical glucocorticoid used was budesonide, a new non-halogenated glucocorticoid with high topical anti-inflammatory activity, similar to that of flunisoloneacetonide, but with a 4–7 times lower systemic activity (19). The daily dosage of 200 µg b.i.d. was the same as that used in previous studies of glucocorticoid mechanisms (12, 13, 14) and in studies concerning patients with seasonal allergic rhinitis, which showed that glucocorticoid was therapeutically efficacious (15).

Histological technique

After the biopsies were taken, the specimens were immediately fixed in ice cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer. After

fixation and dehydration, the specimens were embedded in EPON and cut with an LKB ultratome at a thickness of 1 µ. From each specimen 1–4 Epon blocks were obtained, and from each block two sections were cut. After sectioning, the specimens were stained for 5 days with toluidine blue at pH 0.5 (0.5% toluidine blue in 0.5 M HCl) for specific visualisation of mast cells (21).

The staining results in a violet metachromasia of mast cell granules and a very faint background staining which facilitates the identification of mast cells, even those containing few granules. In two sections from each block, all mast cells with nuclei in the plane of the sections were counted. The area of each section was then measured with planimetry with the help of a digitizer (HiPad, Houston Instruments) served by a microcomputer (Luxor ABC 80, Sweden). The number of mast cells per mm² was then estimated. Due to the small section thickness in relation to mast cell size, this figure gives a valid approximation of the mast cells per unit volume of tissue.

STATISTICAL ANALYSIS

The significance of the difference in the number of mast cells per mm² before and after treatment was determined by means of Student's *t*-test. Analysis of variance was applied as a reproducibility test in the double observations from each specimen.

RESULTS

The use of identical forceps resulted in rather uniform biopsy specimens with a depth of 2 mm from the epithelium, comprising almost the entire thickness. The mast cells were easily identified with the stain used, and individual granules were readily discerned. The cells were irregularly shaped and contained relatively few granules compared with connective tissue mast cells. The mast cells were often found in association with blood vessels but did not appear to have any specific structural relation to mucous glandular tissue. The mast cells were rather evenly distributed in the subepithelial layers and

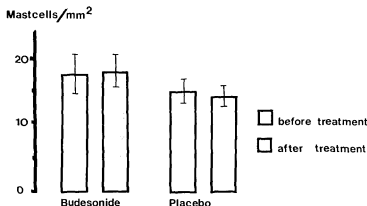


Fig. 1. Number of mast cells per mm² of section area $\pm$ SEM. The changes are not statistically significant.

exceedingly rare within the epithelium. In the entire material studied, only two single mast cells were found within the epithelium.

In the light microscope, no qualitative changes of the mast cells in terms of size, shape, and number of granules were observed. The granules were confined to the cells in the tissues from the treated as well as the non-treated patients. No signs of mast cell degranulation were found.

The mast cells were counted in two sections from each biopsy specimen. The reproducibility of the counting procedure was tested by an analysis of variance. The mean number of mast cells lay between 15–20/mm² (Fig. 1), and the variance between pairs 1.44 (S^2) indicated a high accuracy of the counting procedure. The results are shown in Fig. 1. The glucocorticoid treatment had not affected the number of mast cells.

DISCUSSION

Topical glucocorticoids have become an established therapy in hay fever. Topical glucocorticoid treatment on the skin may induce dermal atrophy; consequently, several studies have been made in order to detect similar effects on the nasal mucosa by examining nasal biopsies before and after treatment for various lengths of time (4, 9). In these studies of perennial rhinitis, no

significant structural changes could be ascertained following glucocorticoid treatment.

Cell quantities are often expressed as number of cells per high power visual field (8). Such values are not comparable between studies, as they are dependent on where the visual fields are placed in the sections. Hence, they are of doubtful significance if the cells are unevenly distributed. In the specimens in the present study the mast cells were practically absent from the epithelium, but they were rather uniformly distributed in the subepithelial layers. The cells in the present study were expressed per unit area in order to facilitate the comparison with other studies.

In a series of studies dealing with the possible effects and mechanisms of action of topical glucocorticoid treatment in allergic rhinitis we have previously been able to show an inhibitory effect on the nasal immediate type allergic reaction, a reduction of histamine content in the nasal mucosa, a blockage of induced histamine release, and a minimal effect of histamine-induced nasal symptoms (12, 13, 14). These effects could be demonstrated in symptom-free hay fever patients after a 1-week treatment period with 400 μ g budesonide nasally.

With regard to mast cells in the mucosa, it is interesting that mucosal mast cells situated in the lamina propria of the intestinal mucosa in rodents constitute a separate cell line with

specific properties; for instance, they are more difficult to visualize, requiring specific fixation and staining technique (21). They proliferate in connection with nematode infections (*Nippostrongylus brasiliensis*) and migrate into the epithelium, where they discharge their granules (1, 5). Nematode infections are also accompanied by high levels of plasma IgE, and the mucosal mast cells seem to be involved in the immunological barrier mechanism of the gut. Similar cells have been observed in human gut mucosa (16, 17). It is not known, however, whether the mucosal mast cells exist as a specific cell type in species other than rats and in different mucosal membranes. Whether mucosal mast cells also constitute a separate cell-line in humans and the nature of their mucous membrane distribution are important questions requiring further studies.

It has been suggested that mucosal mast cells in the nose, as well as basophils in allergic rhinitis, migrate to and through the epithelium and that the number of these cells within the epithelial lining and on the surface of the mucosal membrane reflects the activity of the allergic disease (2, 3). Since the patients in the present study were asymptomatic, the finding of a mere two single mast cells within the epithelium corresponds well with this conception.

Topical corticosteroid treatment has also been shown to reduce the number of mediator cells on and in the surface of the nasal mucosa (11) when symptoms were present. In the present study, dealing with symptom-free patients, no mast cell changes could be observed. From this it can be concluded that the inhibitory effect on the immediate allergic reaction, and the reduction of histamine in the nasal mucosa found previously in symptom-free patients (12, 14), were not due to a reduction of the number of mast cells. The reduction of histamine content might be due to a reduction of the histamine-storing capacity of the cells. However, there were no signs of degranulation or reduction of the density of granules in the mast cells. Light microscopy methods are inadequate for a study of the finer structural alteration of the mast

cells; this calls for electron microscopy. Such studies are in progress.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Professor Lennart Enerbäck for help with the histological procedures, and for valuable discussions concerning mast cells, as well as to Docent Hans Forngren for the selection of patients.

This study was supported by grants from the Swedish Medical Research Council project No. 2235 and from AB Draco, Lund, Sweden.

REFERENCES

1. Enerbäck, L.: The gut mucosal mast cell. *Monogr. Allergy* 17, 222–232, 1981.
2. Hastie, R., Heroy, J. H. & Levy, D.: Basophil leukocytes and mast cells in human nasal secretions and scrapings studied by light microscopy. *Lab. Invest.* 40, 554–561.
3. Kawabori, S., Unno, T., Okuda, M. & Otsuka, H.: Electron microscopic studies of basophilic cells in allergic nasal secretions and mucous membranes. *Rhinology* 19, Suppl. 1, 115–123, 1981.
4. Kleini, J. P., Virolainen, E. & Puhakka, H.: The effect of intranasal beclomethasone dipropionate on the nasal mucosa. *Rhinology* 18, 19–24, 1980.
5. Miller, H. R. P.: The structure and function on mucosal mast cells. *Biol. Cell* 39, 229–232, 1980.
6. Mygind, N. & Clark, T. H. J.: Topical steroid treatment in asthma and rhinitis. Ballière Tindall, London, 1980.
7. Mygind, N.: Mucosal changes in the airways during long-term corticosteroid treatment. *Eur. J. Respir. Dis.* 63, Suppl. 122, 229–234, 1982.
8. Mygind, N., Viner, A. S. & Jackman, N.: Histology of nasal mucosa in normals and in patients with perennial rhinitis. *Rhinology* 12, 131–136, 1974.
9. Mygind, N., Sörensen, H. & Pedersen, C. B.: The nasal mucosa during long term treatment with beclomethasone dipropionate. *Acta Otolaryngol. (Stockh.)* 85, 437–443, 1978.
10. Okuda, M. & Senba, O.: Effects of beclomethasone dipropionate nasal spray on subjective and objective findings in perennial rhinitis. *Clin. Otolaryngol.* 53, 15–21, 1980.
11. Okuda, M., Ohsuka, H. & Sakaguchi, K.: Effects of anti-allergic drugs on nasal allergy. Abstracts from XII World Congress of Otorhinolaryngology, Budapest, Hungary, p. 117, 1981.
12. Pipkorn, U.: Budesonide and nasal allergen challenge testing in man. *Allergy* 37, 129–134, 1982.
13. Pipkorn, U.: Budesonide and nasal histamine challenge. *Allergy* 37, 359–363, 1982.
14. Pipkorn, U. & Andersson, P.: Budesonide and nasal mucosal histamine content and anti IgE induced histamine release. *Allergy* 37, 591–595, 1982.

15. Pipkorn, U. & Rundercrantz, H.: Budesonide and beclomethasone dipropionate in hay fever - a single blind comparison. *Eur. J. Respir. Dis.* 63, Suppl. 122, 211-220, 1982.
16. Ruitenbergh, E. J., Gustowska, L. & Ruitenbergh, H. M.: Effect of fixation on the light microscopical visualization of mast cells in the mucosa and connective tissue of the human duodendum. *Int. Arch. Allergy Appl. Immunol.* 67, 233-238, 1982.
17. Strobel, S., Miller, H. R. P. & Ferguson, A.: Human intestinal mucosal mast cells: evaluation of fixation and staining techniques. *J. Clin. Pathol.* 34, 851-858, 1982.
18. Svedmyr, N.: Effects of glucocorticoids in the airways. *Eur. J. Respir. Dis.* 63, Suppl. 122, 48-53, 1982.
19. Thalén, A. & Brattsand, R.: Synthesis and anti-inflammatory properties of budesonide, a new non-halogenated glucocorticoid with local activity. *Arzneim.-Forsch./Drug Res.* 29, 1687-1690, 1979.
20. Vilsvik, J. S., Jenssen, A. O. & Walstad, R.: The effect of beclomethasone dipropionate aerosol on allergen induced nasal stenosis. *Clin. Allergy* 5, 291-294, 1975.
21. Wingren, U. & Enerbäck, L.: Mucosal mast cells of the rat intestine: a reevaluation of fixation and staining properties, with special reference to protein blocking and solubility of the granular glucosamines. *J. Histochem.* (Accepted for publication) 1982.

Address:
Ulf Pipkorn
ENT Department
Sahlgrens Hospital
S-413 45 Göteborg
Sweden

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.