Effect of a prolonged topical glucocorticosteroid on interleukin-5 
production and eosinophilic recruitment in the nasal submucosal 
compartment

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Aim

Intranasal corticosteroids offer effective treatment for allergic rhinitis. The action of interleukin 5 (IL-5) (Th2-type cytokine) and its response to intranasal steroids has not been thoroughly studied in the deep compartment of the nasal mucosa. The aim of this study was to determine the influence of prolonged topical glucocorticosteroid on the allergic inflammatory responses in the deep compartment of the nasal mucosa in patients with allergic rhinitis.

Materials and methods

Fluticasone furoate spray was used once daily. Biopsies were obtained from 22 patients with perennial allergic rhinitis at different intervals: before treatment with nasal corticosteroids, and after 1, 6, and 12 months. Biopsies were taken from 18 individuals serving as a control group. All biopsies were examined by light microscopy and immunohistochemistry.

Results

The results showed the efficacy of fluticasone in reducing the number of eosinophils in both epithelial and subepithelial layers, which suppresses the allergic manifestations. The maximum reduction occurred after 12 months. This is achieved by reducing the number of eosinophils and IL-5 in both epithelial and subepithelial compartments.

Conclusion

Intranasal corticosteroids effectively reduce both the number of eosinophils and IL-5 expression inside activated eosinophils. They influence both the epithelium and the deep compartment of the nasal mucosa.

Keywords:
allergic rhinitis, eosinophils, fluticasone furoate, immunohistochemistry, interleukin 5, Th2-type cytokine

Introduction

Allergic rhinitis is increasing in prevalence worldwide, resulting in morbidity and complications in all age groups. Intranasal corticosteroids may provide effective treatment. Accurate understanding of the mechanism of action of corticosteroids on cytokines and the eosinophilic influx can minimize the morbidity associated with allergic rhinitis. To our knowledge, the action of interleukin 5 (IL-5) (Th2-type cytokine) has not been studied thoroughly in the deep compartment of the nasal mucosa in patients with perennial allergic rhinitis and the influence of prolonged topical steroids on the secretion of this cytokine in vivo is yet to be determined. The aim of this study was to determine the influence of prolonged topical glucocorticosteroid on the allergic inflammatory responses in the deep compartment of the nasal mucosa in patients with perennial allergic rhinitis.

Nasal mucosal infiltration by inflammatory cells is a constant feature of allergic rhinitis. The inflammatory cells secrete mediators and cytokines that are responsible for the clinical pictures of the disease. Ultimately, this results in a significant increase in the number of activated helper T-cells (CD25 +) [1]. Antigen challenge results in the increased expression of the Th2 cytokines (IL-4, IL-5, and GM-CSF) in patients with allergic rhinitis. Eosinophils also increase in number in both the nasal secretions and the nasal mucosa after allergen challenge or exposure [2]. Recent studies have provided information about the development and recruitment of eosinophils from the bone marrow into respiratory epithelium. Various studies have shown that nasal secretions, obtained by lavage or scraping, and the submucosa, sampled by biopsy are different in terms of cellular accumulation. The submucosa has more lymphocytes than eosinophils and vice versa in the superficial compartment [3–5]. Topical corticosteroids have been shown to be highly efficient in the treatment of allergic rhinitis. Local steroids have a rapid onset; within 3 h of its topical application, nasal allergic symptoms are reduced [4,6]. It is widely assumed that apoptosis of eosinophils is a central component of resolution of allergic airway disease. The effect of topical corticosteroids on different compartments of the nasal mucosa and the mechanism by
which these agents inhibit allergen-induced cellular influx remains controversial [7,8]. In studies that used allergen challenge in patients with seasonal allergic rhinitis, after 1 or 6 weeks of topical steroid treatment, inhibition of these pleiotropic proeosinophilic cytokines and downregulation of the eosinophilic count were observed both in the nasal secretions and in the submucosa [6,9,10]. Other studies have provided different information on the effect of topical corticosteroids on the different nasal mucosal compartments in patients with perennial rhinitis, where pretreatment with beclomethasone for 3 months reduced eosinophils in the nasal secretions, but not in the lamina propria, lending credibility to the hypothesis that intranasal steroids might be more active on the superficial compartment of the nasal mucosa than on deeper compartments [1,11–13].

There are very few experimental clinical models that readily unravel therapeutic potency of airway steroids as a maintenance treatment for periods more than 3 months in perennial (persistent) allergic rhinitis. Thus, little is known about the added therapeutic gain after the establishment of clinical improvement. Therefore, we planned this study in an attempt to verify the effect of prolonged topical corticosteroids on eosinophilia and chemokine production in the nasal submucosal compartment.

Materials and methods
Biopsies were obtained from 22 patients with perennial allergic rhinitis at the Department of Otorhinolaryngology at Fayoum University Hospital. The ethical committee at the University Hospital approved the study and all patients provided their written informed consent before inclusion in the study. None of the patients received intranasal corticosteroids, antihistamines or antileuko- trienes, oral and intranasal decongestants, or intranasal anticholinergics within 2 weeks before the study. No Patient received oral and/or intramuscular corticosteroids within 4 weeks before the baseline biopsy. Eighteen individuals without perennial allergic rhinitis or any form of atopy served as a control group.

Local fluticasone furoate spray (GlaxoSmithKline, Brentford, Middlesex, UK) was used as a local nasal spray once daily. The starting dose was 110 mcg (two puffs per nostril) for patients 12 years of age and older. When symptoms had been controlled, the dosage was reduced to 55 mcg (one puff in each nostril once daily).

Biopsy and tissue preparation
The nasal mucosa at the anterior tips of the inferior turbinates was decongested with oxymetazoline and anesthetized topically with pontocaine. Next, 0.5–1.0 ml of 1% lidocaine in 1:200 000 epinephrine was injected. Nasal biopsies with dimensions averaging 4 × 3 mm were obtained from the anterior tips of the inferior turbinates with a punch biopsy forceps as follows: before treatment with nasal corticosteroids, and after 1, 6 months, and 1 year of intranasal corticosteroid. Bleeding after biopsy was controlled by the local application of silver nitrate. Biopsies were also taken from the 18 individuals serving as the control group.

Microscopic assessment of biopsy specimens
All tissue sections were examined using an Olympus light microscope (Olympus America Inc., Center Valley, Pennsylvania, USA) by two independent observers blinded to the experimental conditions. The numbers of eosinophils were counted in the epithelium and in the adjacent lamina propria in 10 randomly selected fields, and the results were expressed as the mean number of positive cells per field.

Immunohistochemistry
Samples were fixed in 4% formaldehyde (phosphate buffered 6.8–7.2; Klinipath) and embedded in paraffin wax before cutting into 5-μm sections and mounting on poly-t-lysine-coated slides. After deparaffinization in parasolve (Prosan), the sections were hydrated through grade ethanol and stained for standard histomorphologic analysis. Immunostaining of specimens was performed using the avidin–biotin complex–alkaline phosphatase technique as follows: the slides were washed with Tris-buffered saline (TBS), assembled with coverplates, and inserted into a cassette (Shandon Coverplate System; Shandon Inc., 171 Industry Drive Pittsburgh, Pennsylvania, USA) to hold them for the rest of the staining. All reagents and buffer solutions were then applied to the slides by pipetting into the coverplate slide assembly. Nonspecific binding sites were blocked with normal goat serum (1:20) for 20 min at room temperature. One hundred microliters of primary Ab or negative control solutions (diluted appropriately with TBS) were then applied and allowed to incubate for 60 min at 37°C. Primary antibodies were then washed with TBS, and 100 μl of biotinylated goat anti-mouse secondary Ab (1:100 diluted in TBS) was applied and allowed to incubate for 45 min at 37°C. After washing with TBS, 100 μl of avidin–biotin–alkaline phosphatase complex was added to the slides and allowed to incubate for 30 min at room temperature. The slides were then washed with TBS, and 100 μl of the brown chromogen solution was added and allowed to incubate in the dark for 20 min at room temperature. The chromogen was then washed with 2 ml of TBS and the slides were placed in staining dishes. They were rinsed briefly in distilled water and counterstained with hematoxylin for 2 min and 15 s. Hematoxylin was washed off the slides by running water for 5 min. The specimens were then dehydrated by brief sequential immersions in 95% alcohol, 100% alcohol and xylene, dried, and coverslips were mounted on the specimens using aqueous mounting medium. Appropriate dilutions of the monoclonal antibodies provided maximal brown staining of the positive cells with little or no background staining. All specimens were stained with each of the monoclonal antibodies in the same assay to eliminate interassay variability. Available nasal specimens with positivity to each of the monoclonal antibodies, as determined by previous staining, were run with each assay and served as positive controls, whereas negative
controls consisted of tissue specimens stained with irrelevant antibodies (IgG1 or IgG2a).

**Results**

The number of eosinophils in the epithelium for specimen B (before treatment) (Fig. 2) was less than or equal to five eosinophils per high-power field (hpf) (× 400). This number decreased in biopsies after 1 month of treatment with corticosteroids, specimen C (Fig. 3) four or less eosinophils per hpf. A greater decrease was observed in biopsies after 6 months of treatment with fluticasone, specimen D (Fig. 4) two or less eosinophils per hpf. A further decrease in the number of eosinophils was observed after treatment for 12 months with fluticasone, specimen E (Fig. 5) only one or less eosinophil per hpf. This was the same count for patients of specimen A (Fig. 1).

For the subepithelial tissue (the deep compartment), similar results were obtained. There was a decrease in the number of eosinophils in specimen E (Fig. 5), two or less eosinophils per hpf, which is equivalent to the count of specimen A (Fig. 1). The results for specimen D (Fig. 4), five or less eosinophils per hpf and specimen C (Fig. 3), eight or less eosinophils per hpf. Whereas the number of eosinophils in specimen B (Fig. 2) was less than or equal to five eosinophils per hpf.

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**Figure 1**

Case of control group (nonallergic patient) showing very occasional eosinophils. IL-5 receptors immunostaining, × 200.

**Figure 2**

A case of an allergic patient (without treatment) showing a large number of eosinophils with a high number of positive (activated) eosinophils (arrows). IL-5 receptors immunostaining, × 200.

**Figure 3**

A case of an allergic patient receiving treatment for 1 month showing a moderate number of eosinophils and a moderate number of activated ones (arrows). IL-5 receptors immunostaining, × 200.

**Figure 4**

A case of an allergic patient receiving treatment for 6 months showing a low number of eosinophils and a low number of activated ones (arrows). IL-5 receptors immunostaining, × 200.

**Figure 5**

A case of an allergic patient receiving treatment for 12 months showing very few number of eosinophils and very few activated ones. IL-5 receptors immunostaining, × 200.
6 and 1 month, respectively. These results further
12 months as compared with specimens obtained after
subepithelial layer in specimens receiving treatment for
phils (total and activated) was maximally reduced in the
preparation administered. Also, the number of eosino-
numbers of both total and activated (positively stained)
was more marked and led to significant reductions in the
nasal biopsy. In the epithelium, the effect of fluticasone
was markedly reduced after treatment with fluticasone
for 12 months compared with specimens obtained after
6 and 1-month duration). We also evaluated the influx of
treatment with fluticasone (12 vs. 6 months
duration at the cellular level, although the allergic rhinitis
symptoms improve much earlier.

Results of immunostaining
The number of cytoplasmic brown staining of eosinophils
was markedly reduced after treatment with fluticasone
for 12 months compared with specimens obtained after
6 and 1 month, respectively.

The intensity of cytoplasmic brown staining of eosinophils
was markedly reduced as we prolonged the dura-
tion of treatment with fluticasone (12 vs. 6 months
and 1-month duration). We also evaluated the influx
of eosinophils in the superficial compartment of the nasal
mucosa [20]. Further studies are required to determine the
manifestations. The reduction in the number of eosino-
phils is inversely proportional to the duration of nasal
corticosteroid treatment. The maximum reduction oc-
curred after 12 months of treatment (Table 1). The least
reduction occurred after treatment for 1 month. The
reduction in specimen B reached values that are similar to
those of specimen A, the control group. The results show
that it takes between 6 months and 1 year of local
corticosteroid treatment to achieve maximum improve-
ment at the cellular level, although the allergic rhinitis
symptoms improve much earlier.

Table 1 The level of eosinophils per high-power filed is
inversely proportional to the duration of local corticosteroid
intake

<table>
<thead>
<tr>
<th>Specimen</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<tr>
<td>X-axis, specimen A–E; Y-axis, average number of eosinophils per high-power field.</td>
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eosinophils in specimen B (Fig. 2) was 10 or less
eosinophils per hpf inspection (x 400) (Figs 1–5).
These results show the efficacy of fluticasone in re-
ducing the number of eosinophils in both epithelial
and subepithelial layers, which suppresses the allergic
manifestations. The reduction in the number of eosino-
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ment at the cellular level, although the allergic rhinitis
symptoms improve much earlier.

Discussion
This study shows that individuals with allergic rhinitis
have a symptomatic nasal phase response involving
markedly elevated epithelial and submucosal eosinophilic
levels with high levels of IL-5 shown by immunohisto-
chemistry. Furthermore, these cytokines are inhibited by
fluticasone administered for long periods. These data are
of interest in part because of the potential importance of
cytokines such as IL-5 in the pathogenesis of allergic
airway disease, and in part because of the lack of
experimental clinical models, in humans, that readily
show the therapeutic potency of airway steroids.

In the present study, markedly increased number of
eosinophils and nasal mucosal IL-5 expressed by eosino-
phils (activated eosinophils) was observed in patients
with allergic rhinitis. This observation is in agreement
with previous reports of Alam et al. [14] and Linden
et al. [15].

The application of fluticasone for long intervals (1, 6, and
12 months) has effectively reduced both the number of
eosinophils and IL-5 expression inside activated eosino-
phils. This was in agreement with the results obtained by
Sim et al. [16], Weido et al. [17], and Masuyama et al. [18],
who reported a significant decrease in cytokine release
(IL-5) coinciding with a clinical improvement. Fuad et al. [3]
reported that intranasal steroid therapy did not
significantly alter the number of total eosinophils or that of
activated eosinophils (IL-5) in the nasal mucosa. The
discrepancy between our results and that of Fuad et al.
can be attributed to several factors. Fuad et al. used the allergen
challenges method and beclometasone dipropionate spray.
In the current study, we did not use allergen challenges and
we used a different preparation, fluticasone furoate [3].
Rak et al. [19] and Lozowicz et al. [20] reported the number
of activated eosinophils (IL-5) to be significantly reduced
in both studies compared with placebo. In the epithelium,
the effect of fluticasone was more marked in both studies
and led to significant reductions in the numbers of both
total and activated eosinophils. These studies, like ours,
support the efficacy of the intranasal steroid, fluticasone,
in reducing allergen-induced eosinophil activation in the nasal
mucosa [20]. Further studies are required to determine the
time it takes for total eosinophils or that of activated
eosinophils (IL-5) to revert to their original levels after the
stoppage of intranasal corticosteroids in allergic patients.

Conclusion
Intranasal corticosteroids effectively reduce both the
number of eosinophils and IL-5 expression inside
activated eosinophils. They influence both the epithelium and the deep compartment of the nasal mucosa.

**Acknowledgements**

**Conflicts of interest**

There are no conflicts of interest.

**References**


