

Nasal cytological changes following pharmacological intervention

Meltzer EO. Nasal cytological changes following pharmacological intervention.

Allergy 1995; 50: 15-20. © Munksgaard 1995.

Symptoms of allergic rhinitis are associated with increased numbers of inflammatory cells in the nasal mucosa. The effects of fluticasone propionate on the nasal mucosal cells of patients with symptomatic allergic rhinitis were evaluated in seven multicentre, double-blind, parallel-group, placebo-controlled, randomised studies. In three seasonal allergic rhinitis studies, significantly more patients receiving fluticasone propionate had a decrease in nasal eosinophils following treatment compared with patients receiving placebo. Similarly, more patients receiving fluticasone propionate had a decrease in nasal basophilic cells, but differences from placebo were not significant in all studies. Nearly identical results were observed in two 24-week perennial allergic rhinitis studies: significantly more patients receiving fluticasone propionate or beclomethasone dipropionate had a decrease in nasal eosinophils compared with patients receiving placebo. Furthermore, a higher percentage of patients receiving corticosteroids also had a decrease in the number of basophilic cells. In two separate seasonal allergic rhinitis studies, significantly more patients receiving fluticasone propionate had a decrease in nasal eosinophils compared with patients receiving terfenadine or astemizole, respectively. The decrease in nasal basophilic cells was also significantly greater with fluticasone propionate compared with astemizole. Inhibition of mediator release from eosinophilic and basophilic cells has also been demonstrated in patients receiving fluticasone propionate compared with patients receiving antihistamines. The results of these studies suggest that the therapeutic benefits of fluticasone propionate aqueous nasal spray in the treatment of seasonal and perennial allergic rhinitis may be related to its ability to reduce nasal mucosal inflammatory cells and to inhibit local mediator activity.

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Key words: allergic rhinitis, seasonal, perennial; fluticasone propionate aqueous nasal spray; eosinophils; basophils.

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Introduction

Allergic rhinitis occurs in individuals with the genetic predisposition to mount in their lymphoid tissue a high and prolonged immunoglobulin E response to airborne allergens. Once sensitised, this response results in the release of preformed and newly generated mediators and the influx of nasal inflammatory cells, including eosinophils, basophilic cells, neutrophils and mononuclear cells (17). These mediators and nasal inflammatory cells cause increased airway hyperresponsiveness, and the symptoms characteristic of allergic rhinitis.

Intranasal corticosteroids interfere with multiple steps in the inflammatory process. They decrease capillary permeability (22), decrease the glandular response to cholinergic stimulation (3), interfere with arachidonic acid metabolism which results in de-

creased mediator production (9), reduce mediator release (21), decrease production of cytokines from T-helper type 2 lymphocytes (11), and inhibit the influx of eosinophils and basophilic cells to the nasal epithelium (4).

In a nasal challenge model, studies at Johns Hopkins University demonstrated an increase in eosinophils and basophilic cells (the majority being basophil leucocytes) following allergen provocation. When the patients were pretreated for 1 week with intranasal corticosteroids, there was a significant reduction in the influx of these cells (4). In previous clinical studies, nasal eosinophilia has been shown to decrease after treatment with beclomethasone dipropionate in patients with seasonal or perennial allergic rhinitis (8). A decrease in eosinophils after treatment with beclomethasone dipropionate has also been observed in nasal scrapings (20). Simi-

larly, another topical corticosteroid, flunisolide, has been associated with decreased nasal eosinophilia in patients with allergic rhinitis (15). Beclomethasone dipropionate has also been reported to inhibit the increase in density of basophilic cells that occurs in the nasal mucosa during the pollen season (7) and to reduce basophilic cells in epithelial scrapings in patients with allergic rhinitis (19).

A series of studies has evaluated the effects of intranasal fluticasone propionate on symptoms of allergic rhinitis and nasal cytology. In all seven studies, significant improvement in nasal symptoms occurred following treatment with fluticasone propionate. This aspect of the studies will not be reviewed; instead, this paper focuses on the effect of intranasal fluticasone propionate on nasal eosinophils and nasal basophilic cells.

Materials and methods

The studies of fluticasone propionate included two 2-week studies of seasonal allergic rhinitis (14, 18), three 4-week studies of seasonal allergic rhinitis (5, 6, 10), and two 24-week studies of perennial allergic rhinitis (2, 23) (Table 1). All were multicentre, double-blind, parallel-group, placebo-controlled,

Table 1. Treatment protocols in seven studies of seasonal allergic rhinitis (SAR) and perennial allergic rhinitis (PAR)

Disease (reference)	Patients	Treatment/Dosage	Duration (weeks)
SAR (14)	n=422	FP ANS 25 µg bd FP ANS 100 µg bd FP ANS 400 µg bd Placebo	2
SAR (18)	n=227	FP ANS 100 µg bd FP ANS 200 µg od Placebo	2
SAR (6)	n=301	FP ANS 100 µg bd FP ANS 200 µg od Placebo	4
SAR (5)	n=348	FP ANS 200 µg od Terfenadine 60 mg bd Placebo	4
SAR (10)	n=304	FP ANS 200 µg od Astemizole 10 mg od Placebo	4
PAR (23)	n=466	FP ANS 100 µg bd FP ANS 200 µg od BDP ANS 168 µg bd Placebo	24
PAR (2)	n=365	FP ANS 100 µg bd FP ANS 200 µg od Placebo	24

FP ANS=fluticasone propionate aqueous nasal spray; BDP ANS= beclomethasone dipropionate aqueous nasal spray; bd=twice daily; od=once daily.

randomised studies. Each study had a run-in period to confirm that the patients enrolled had moderate to severe symptoms of allergic rhinitis. After treatment for 2 or 4 weeks in the seasonal allergic rhinitis studies and 24 weeks in the perennial allergic rhinitis studies, patients were evaluated at a follow-up visit.

Nasal cytology specimens were obtained at the beginning and end of the treatment period. There was no cytologic inclusion criterion in the seasonal allergic rhinitis studies but all patients were required to have documented nasal eosinophilia to participate in the perennial allergic rhinitis studies. All studies were approved by an institutional review board and all patients gave signed informed consent.

The nasal mucosal sample was collected under direct vision after the patient cleared the nose of excess secretion. The cupped tip of a disposable Rhinoprobe (Apotex Scientific, Inc., Arlington, TX, USA) was gently pressed on the mucosal surface of the medial aspect of the inferior turbinate. Two or three short scrapes of the epithelial membrane were made to obtain the sample. The specimen was spread on a plain slide and immediately fixed for at least 1 min in 90% ethyl alcohol. After draining the excess alcohol, the slide was dipped sequentially in Wright-Giemsa stain (Volu-Sol, Logos Scientific, Inc., Henderson, NV, USA) for 15 s, in Volu-Sol buffer for 30 s and in Volu-Sol haematology rinse for 5 s. The slide was drained of excess fluid between procedures. After air drying, the cytogram was examined under oil immersion by light microscopy. The grading was semiquantitative on a scale of 0 to 4+, as presented in Table 2 (12). Cytograms within each study were examined by one microscopist.

Results

The first study of seasonal allergic rhinitis included over 200 patients with evaluable paired pre- and post-treatment nasal mucosal specimens (14). At base-line, approximately 80% of patients had eosinophils, 40% had basophilic cells and over 60% had neutrophils in their nasal samples. After 2 weeks of treatment, there were significantly fewer patients in the fluticasone propionate groups (25 µg twice daily, 100 µg twice daily, or 400 µg twice daily) compared with the placebo group with a score of at least 1+ for eosinophils and basophilic cells. There were also significantly more patients in the active treatment groups with decreases in the number of eosinophils and basophilic cells and this appeared to be dose-related (Fig. 1). This is the only study which also showed a decrease in nasal neutrophils. This occurred in patients receiving the two higher doses of fluticasone propionate.

In the second seasonal allergic rhinitis study, all patients in the fluticasone propionate groups re-

ceived the same total daily dose (18). However, some patients received 100 µg twice daily and some received 200 µg once daily in order to document any differences in outcome due to dosing frequency. At base-line, similar percentages of patients with each cell type were present in the active and placebo groups. Although the placebo group showed no change over 2 weeks, there was a significant decrease in the percent of patients with eosinophils in both of the fluticasone propionate treatment groups. The decrease in the 100 µg twice daily group was from 83% to 32% of patients and in the 200 µg once daily group was from 76% to 43%. The percent of patients with basophilic cells was significantly decreased in the twice daily fluticasone propionate group but not in the once daily group.

In the third study, 153 patients had evaluable paired pre- and post-treatment nasal mucosal specimens (6). In agreement with the observed improvement in nasal symptoms, after 4 weeks of treatment eosinophils were decreased in more than 60% of patients receiving fluticasone propionate 100 µg twice daily or 200 µg once daily compared with only 25% of patients receiving placebo (*P* < 0.001). Basophilic cells were reduced in 49% of patients in the fluticasone propionate 100 µg twice daily group compared with 18% of patients in the placebo group (*P* < 0.001). Basophilic cells were reduced in 38% of

Table 2. Grading of nasal cytograms

Quantitative scoring	Semiquantitative scoring	Grade
Eosinophils*, neutrophils*		
0.1–1.0	None	0
1.1–5.9	Few scattered cells	1+
6.0–15.9	Moderate number of cells and small clumps	2+
16.0–20.0	Large clumps of cells which do not cover the entire field	3+
> 20.0	Large clumps of cells cover the entire field	4+
Basophilic cells*		
0.1–0.3	None	0
0.4–1.0	Few scattered cells	1+
1.1–3.0	Moderate number of cells and small clumps	2+
3.1–6.0	Large clumps of cells which do not cover the entire field	3+
> 6.0	Large clumps of cells cover the entire field	4+
Goblet cells†		
0–24%	None or a few cells	1+
25–49%	Moderate number	2+
50–74%	Large number	3+
75–100%	Large number, may cover the entire field	4+
Bacteria		
N/A	None	0
N/A	Occasional	1+
N/A	Moderate number	2+
N/A	Large number	3+
N/A	Large number covers the entire field	4+

* Mean of cells per 10 high power fields (x 1000).
† Ratio of goblet cells to epithelial cells, expressed as percent.

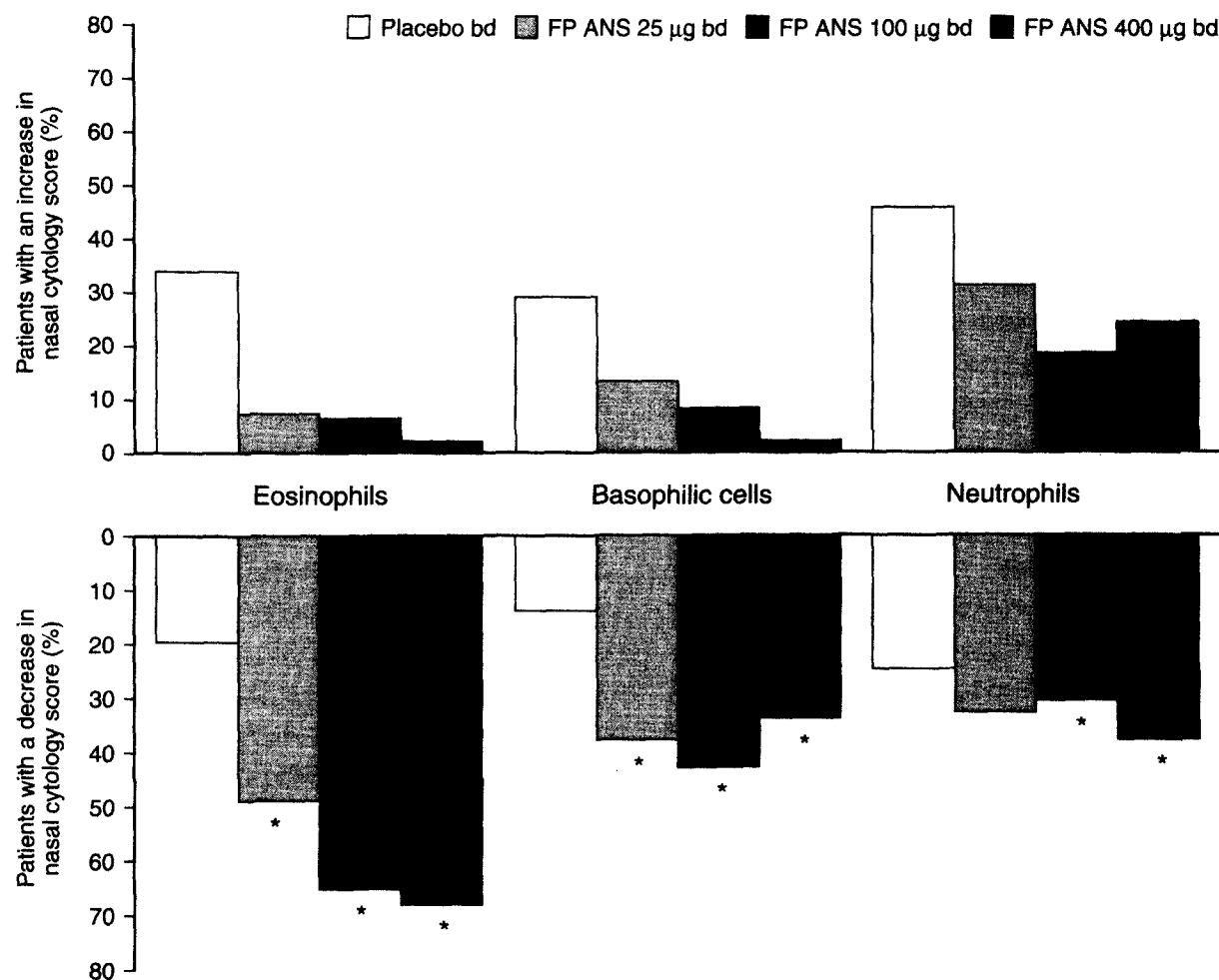


Fig. 1. Percent of patients with seasonal allergic rhinitis with increases or decreases in eosinophils, basophilic cells, and neutrophils among those with evaluable paired pre- and post-treatment nasal mucosal specimens.* *P* < 0.05 fluticasone propionate aqueous nasal spray (FP ANS) vs placebo. bd = twice daily. Adapted from Meltzer et al. 1990 (14).

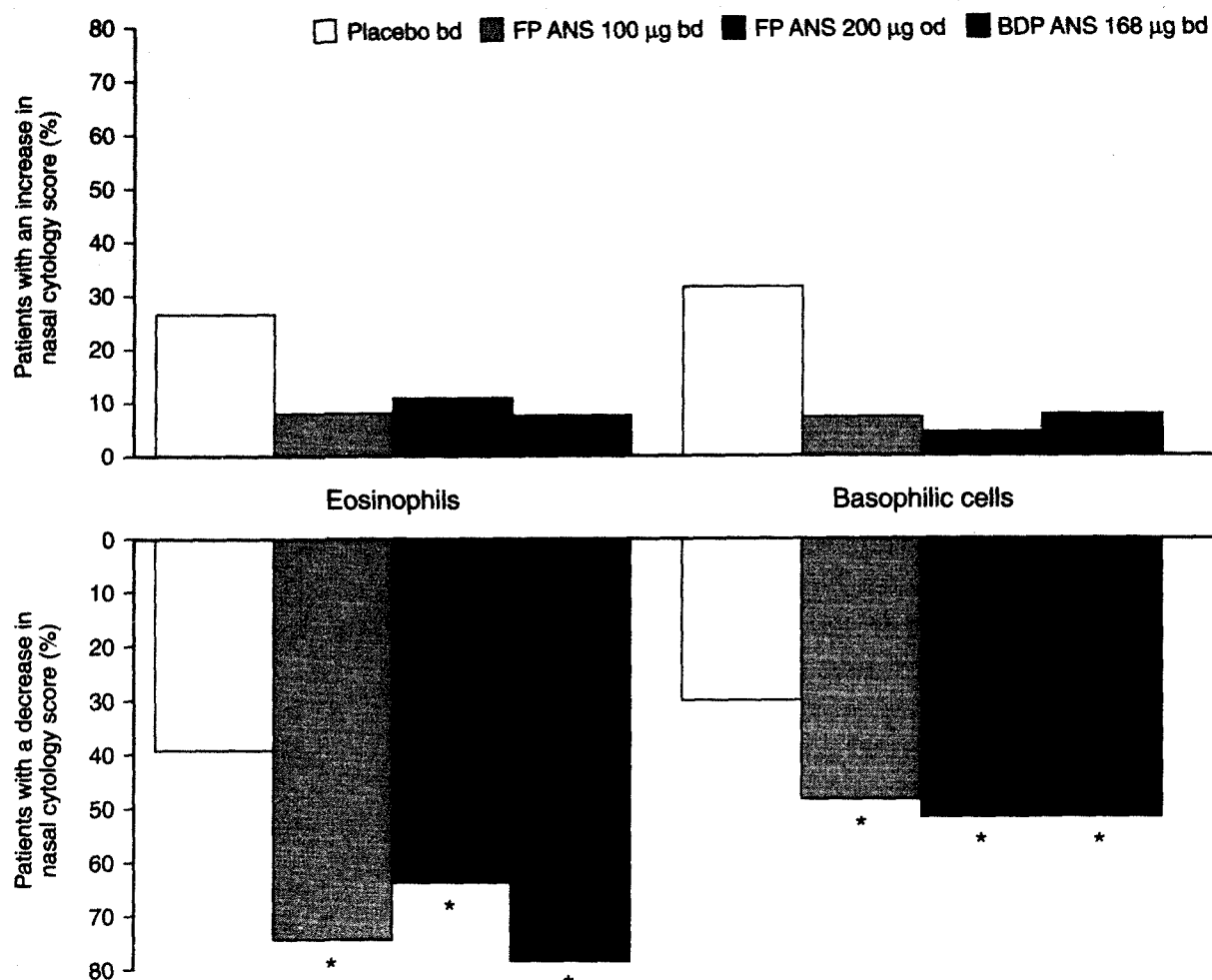


Fig. 2. Percent of patients with perennial allergic rhinitis with increases or decreases in eosinophils and basophilic cells among those with evaluable paired pre- and post-treatment nasal mucosal specimens. * $P < 0.05$ active treatment vs placebo. FP ANS = fluticasone propionate aqueous nasal spray; BDP ANS = beclomethasone dipropionate aqueous nasal spray; bd = twice daily; od = once daily. Adapted from van As et al. 1993 (23).

patients in the fluticasone propionate 200 µg once daily group, but this difference did not reach statistical significance when compared with placebo.

Intranasal fluticasone propionate has been compared with an oral antihistamine in patients with seasonal allergic rhinitis in two placebo-controlled studies. In the first study, patients received either intranasal fluticasone propionate 200 µg once daily or terfenadine 60 mg twice daily for 4 weeks. More patients treated with intranasal fluticasone propionate exhibited decreases in nasal eosinophils over the course of 1 month than those receiving placebo ($P < 0.05$) (5). However, patients receiving terfenadine did not differ from placebo in this regard. The change in ratings for numbers of nasal basophilic cells was not significantly different between the groups.

In a similarly designed study, intranasal fluticasone propionate 200 µg once daily was compared with astemizole 10 mg once daily for 4 weeks (10). Fewer patients receiving fluticasone propionate had nasal eosinophils following treatment compared with patients receiving astemizole or placebo ($P < 0.05$). In addition, the percent of patients with a decrease in nasal basophilic cells was significantly greater with the intranasal corticosteroid compared with the oral antihistamine ($P < 0.05$).

Two studies in perennial allergic rhinitis have also

included nasal cytologic evaluations. All patients were required to have nasal eosinophilia at base-line. In the first study, after 24 weeks of treatment, nasal eosinophilia was decreased in 64–79% of patients treated with fluticasone propionate 100 µg twice daily, 200 µg once daily, or beclomethasone dipropionate 168 µg twice daily compared with 39% of patients receiving placebo ($P < 0.05$) (Fig. 2) (23). There were no significant differences between fluticasone propionate or beclomethasone dipropionate. Similarly, more patients receiving active treatment had decreased numbers of nasal basophilic cells compared with placebo ($P < 0.05$) (Fig. 2). In the second study, nasal eosinophils were reduced or eliminated in more patients treated with intranasal fluticasone propionate 100 µg twice daily or 200 µg once daily compared with placebo ($P < 0.05$) (2). The second study did not include a beclomethasone dipropionate treatment group. Nasal basophilic cell decreases tended to be greater in the active treatment groups but, compared with placebo, differences did not reach statistical significance.

A review of the first three seasonal allergic rhinitis studies in approximately 500 evaluable patients reveals that, at base-line, approximately 80% of patients had eosinophils, 47% had basophilic cells and 71% had neutrophils in their nasal mucosal specimens. Following treatment with intranasal

fluticasone propionate, nasal cytology scores of 1+ were found in 40% of patients for eosinophils, 23% for basophilic cells and 63% for neutrophils. In contrast, in patients receiving placebo, nasal cytology scores of 1+ were found in 76%, 37% and 73% of patients for eosinophils, basophilic cells and neutrophils, respectively. There were also greater changes in the percentages of patients with decreases in nasal inflammatory cells following treatment with fluticasone propionate. Approximately 62% of patients had decreases in eosinophils, 38% had decreases in basophilic cells, and 36% had decreases in neutrophils. In contrast, in patients receiving placebo, the percentage with decreases was 23% for eosinophils, 21% for basophilic cells, and 23% for neutrophils.

The two perennial allergic rhinitis studies when combined provide similar results. At base-line, all patients were required to have nasal eosinophilia. Furthermore, basophilic cells were seen in 55% and neutrophils in 93% of patients. Following intranasal corticosteroid therapy, these cells were present in only 41%, 12% and 71% of patients, respectively (16). A greater percentage of patients receiving active treatment compared with placebo also had decreases in scores for the various cells. Eosinophils decreased in 70%, basophilic cells in 51%, and neutrophils in 44% of patients treated with intranasal fluticasone propionate or beclomethasone dipropionate. In patients receiving placebo, eosinophils were decreased in 50%, basophilic cells in 30%, and neutrophils in 43% of patients.

Discussion

The results of the clinical and cytologic components of these studies suggest that the symptoms of allergic rhinitis are associated with an influx of nasal inflammatory cells including eosinophils, basophilic cells and neutrophils. Following treatment with fluticasone propionate aqueous nasal spray, at doses ranging from 25 µg twice daily to 400 µg twice daily for 2 or 4 weeks in seasonal allergic rhinitis studies and 24 weeks in perennial allergic rhinitis studies, symptoms significantly improved compared with placebo. Fluticasone propionate aqueous nasal spray was associated with a significant decrease in eosinophils with every dose studied, with a dose response trend; a significant decrease, or a trend to decrease, in basophilic cells; and a trend to decrease in neutrophils. Similar changes occurred after beclomethasone dipropionate treatment (168 µg twice daily) for 24 weeks in perennial allergic rhinitis. These findings suggest that the therapeutic benefits of intranasal fluticasone propionate and beclomethasone dipropionate are reflected in, and may be caused by, the decrease in nasal inflammatory cells.

Although the absolute reduction in eosinophils

and basophilic cells as a result of intranasal fluticasone propionate appears to be important for the treatment of allergic rhinitis, it may be the decrease in intracellular mediators of these cells that provides most benefit. Studies have demonstrated the effects of eosinophil and basophilic cell granules and their membrane-associated products in inflammatory and allergic disorders. The eosinophil, for example, has multiple mediators which can damage the respiratory epithelium. These include major basic protein, eosinophil peroxidase, eosinophil cationic protein, and eosinophil-derived neurotoxin. Eosinophils also produce leukotrienes and platelet activating factor, resulting in increased vasodilation, increased vascular permeability, and oedema. The eosinophil can also enhance the release of histamine and other mediators from mast cells and basophilic cells.

To document the effect of intranasal fluticasone propionate on eosinophils and eosinophil mediators, patients with allergic rhinitis were treated with either fluticasone propionate, astemizole or placebo (1). Symptom improvement was significantly greater in the corticosteroid group ($P < 0.05$). Levels of nasal eosinophils, eosinophil-derived neurotoxin and eosinophil survival-enhancing cytokines (interleukin-5, interleukin-3 and granulocyte-macrophage colony stimulating factor) were significantly lower after 2 weeks of treatment with fluticasone propionate compared with astemizole or placebo ($P < 0.05$).

In a separate study, the effects of intranasal fluticasone propionate on basophilic cells and their mediators were compared with those of terfenadine or placebo in patients with seasonal allergic rhinitis ($n = 43$) (13). Compared with placebo and the antihistamine, nasal symptoms were significantly improved in the corticosteroid group (5). Although there were no significant differences in the number of basophilic cells between groups, the levels of histamine and tryptase were significantly lower following 4 weeks of treatment with fluticasone propionate compared with terfenadine or placebo. The findings in this and the immediately preceding study suggest that the therapeutic benefits of intranasal fluticasone propionate may be related to its ability to inhibit production, release, or activity of local mediators from eosinophils and basophilic cells, in addition to causing a decrease in the number of these nasal inflammatory cells.

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