

# Effect of intranasal fluticasone propionate on the immediate and late allergic reaction and nasal hyperreactivity in patients with a house dust mite allergy

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## Summary

**Background** Patients with perennial allergic rhinitis develop nasal symptoms not only after allergen exposure, but generally also after non-specific stimuli.

**Objective** To evaluate the effect of 2 week's treatment with fluticasone propionate aqueous nasal spray (FPANS) on the nasal clinical response, inflammatory mediators and nasal hyperreactivity.

**Methods** Twenty-four rhinitis patients allergic to house dust mite (HDM), participated in a double-blind, placebo-controlled crossover study. After 2 week's treatment with placebo or 200 µg FPANS twice daily, patients were challenged with HDM extract. Symptoms were recorded and nasal lavages were collected for up to 9.5 h after challenge. Nasal hyperreactivity was determined by histamine challenge 24 h later.

**Results** Because of a carry-over effect for the immediate symptom score, for this variable only the data from the first treatment period were used. FPANS treatment resulted in a significant decrease of nasal symptoms with 70%, 69% and 63% after 100, 1000 and 10 000 Biological Units (BU)/mL of HDM extract respectively. Active treatment resulted in a 76% decrease of the late-phase symptoms. FPANS treatment significantly reduced albumin influx after HDM 1000 BU/mL with 62% and tended to reduce tryptase release after HDM 1000 BU/mL ( $P=0.0629$ ). During the late phase FPANS treatment reduced albumin influx with 67% and eosinophil cationic protein (ECP) release with 83%. No effect of FPANS was seen on histamine levels. FPANS significantly decreased histamine-induced symptom score with 34%, secretion with 32% and sneezes with 41%.

**Conclusion** FPANS significantly inhibits the immediate and late allergic response, and nasal hyperreactivity, probably by suppressing mast cells and eosinophils in the nasal mucosa.

**Keywords:** fluticasone propionate, perennial allergic rhinitis, house dust mite, nasal lavage, inflammatory mediators, early allergic response, late allergic response, nasal hyperreactivity

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## Introduction

Allergic rhinitis is a common problem (prevalence ranging from 2 to 20 % [1]), characterized by symptoms such as sneezing, itching, rhinorrhoea and nasal congestion. The pathophysiology is associated with the presence of inflammatory cells such as eosinophils, mast cells, T cells and Langerhans cells [2–4] in the nasal mucosa. After re-exposure to allergen, mast cells are activated by cross-linking of allergen to mast cell-bound specific immunoglobulin E (IgE). The activated mast cells release vasoactive mediators such as histamine and leukotrienes related to the early nasal symptoms, and chemotactic mediators that attract inflammatory cells such as eosinophils associated with the late-phase response [5].

Connell [6] described a phenomenon known as nasal priming: repetitive exposure to allergen causes an increased sensitivity to allergens. This effect was confirmed by others [7,8]. Exposure to allergen also increases nasal responsiveness to non-specific stimuli [9]. However, the pathophysiology of nasal hyperreactivity and nasal priming is still unclear.

In the lower airways, the late-phase reaction was found to be associated with inflammation and bronchial hyperreactivity [10–12], suggesting that inflammation is involved in the pathogenesis of hyperreactivity. These associations could not be confirmed in nasal challenge studies performed in patients with a pollen allergy [13–15], which makes the pathogenetic role of inflammation in nasal hyperreactivity less plausible.

However, in a study with rhinitis patients allergic to house dust mite (HDM), Gerth van Wijk [16] has shown an association between nasal responsiveness to allergen and pre-existent nasal hyperreactivity, a finding more in agreement with data from the lower airways.

Anti-inflammatory therapy with intranasal corticosteroids has extensively been shown to be effective in the treatment of seasonal and perennial rhinitis [17]. It has been shown that topical corticosteroids have an effect on both the early and late-phase reaction after allergen challenge, on nasal priming and on nasal hyperreactivity in patients allergic to pollen [18,19]. In the present study we want to evaluate the anti-inflammatory effects of topical corticosteroid therapy on nasal response to HDM challenge, and on allergen-induced nasal hyperreactivity. In an attempt to provide further differentiation between the local anti-inflammatory effects and the unwanted systemic effects of corticosteroids, a new, topically active, fluorinated glucocorticosteroid: fluticasone propionate has been developed. It has shown to have twice the potency of beclomethasone dipropionate and to have minimal systemic effects [20,21] because of extensive first-pass metabolism by the liver.

In 24 rhinitis patients allergic to HDM the effect of 2 week's treatment with fluticasone propionate on nasal response to HDM challenge and nasal hyperreactivity was studied. Nasal response to allergen was monitored by symptoms and mediators in nasal lavage fluid (histamine, albumin as marker for vasopermeability, tryptase as mast-cell marker and ECP as marker for activated eosinophils) for up to 9.5 h.

## Methods

### Subjects

Twenty-four rhinitis patients, 11 women and 13 men aged 21–50 years (mean 34 years) participated in this study. They had to fulfil the following criteria: a history of perennial rhinitis; intradermal skin reaction of at least 1 plus-sign to 3 BU/mL HDM extract, (ALK Benelux, Groningen, the Netherlands), according to the standardized plus-sign scoring system defined by Norman [22]; aged between 15 and 65 years. The only patient with a concomitant pollen allergy was tested outside the pollen season. Patients with concomitant allergy to pets were only included when they had no contact with pets.

To prevent interference with FPANS, symptomatic medication for rhinitis had to be withdrawn. Oral corticosteroids had to be withdrawn 2 months, astemizole 6 weeks, nasal or inhaled corticosteroids, cromoglycate and nedocromil 3 weeks and antihistamines 3 days before entering the study. Patients with nasal polyposis, nasal surgery less than 3 months before the study, nasal infection during 2 weeks before the study or immunotherapy in the past were excluded.

### Study design

In 24 patients with perennial rhinitis a double-blind placebo-controlled crossover study was performed. Patients were treated with placebo or 200 µg FPANS twice daily (i.e. twice the recommended dosage) for 2 weeks. At visit 1 patients received a nasal spray containing 50 µg fluticasone propionate/actuation or placebo. After the first treatment period, followed by a wash-out period of 3 weeks, they received the complementary nasal spray (visit 4). After either treatment period patients were challenged with HDM extract. Symptom scores were recorded and nasal lavages were performed for up to 9.5 h after HDM challenge (visit 2 and 5). Allergen-induced nasal hyperreactivity was determined by nasal histamine provocation 24 h after HDM challenge (visit 3 and 6).

During the study patients were not allowed any medication affecting nasal function other than terfenadine

60 mg, which was provided as escape-medication. Terfenadine had to be withdrawn 3 days before the nasal challenges were performed.

The study was performed during the period January–August, to minimize natural exposure to HDM. The study was approved by the Medical Ethical Committee of the University Hospital Rotterdam-Dijkzigt and all patients gave written informed consent.

#### *Nasal challenge with HDM and histamine*

Challenges were performed in accordance with the methods described by Gerth van Wijk [13, 16]. On each occasion the patients waited for 30 min before the test to give the nasal mucosa time to acclimatize.

Nasal challenges with HDM extract (100 BU/mL, 1000 BU/mL, 10000 BU/mL, ALK Benelux, Groningen, the Netherlands) were performed at 10-min intervals after challenge with phosphate-buffered saline (PBS), containing human serum albumin 0.03% and benzalkonium chloride 0.05% (ALK Benelux, Groningen, the Netherlands). The HDM extract was sprayed into each nostril by means of a nasal pump spray delivering a fixed dose of 0.125 mL solution. Nasal response was monitored 10 min after each challenge, and hourly from 0.5 until 9.5 h after the last challenge. Nasal responsiveness was monitored by the number of sneezes, the amount of secretion collected according to Borum [23] and a symptom score according to Lebel [24].

Nasal challenge with histamine phosphate (0.25, 0.50, 1.0, 2.0 and 4 mg/mL) was performed at 5 min intervals after challenge with PBS. The amount of secretion, the number of sneezes and the symptom score according to Lebel [24] were used as nasal-response indicators.

#### *Nasal lavage*

Nasal lavages were performed as described by Naclerio [25]. This protocol comprises four pre-challenge washings with isotonic saline solution to clear the nose from secretions and to obtain baseline levels of mediators. In order to prevent nasal congestion due to allergen challenge, oxymetazoline 0.1% (two 0.125 mL puffs) was applied in both nostrils. After 5 min the oxymetazoline was washed out just prior to the nasal challenge with PBS. A nasal lavage was performed 10 min after PBS and after each allergen challenge, immediately before the subsequent challenge. Subsequently, lavage fluid was obtained hourly from 0.5 to 9.5 h after the last allergen challenge. Nasal lavages were performed with 10 mL isotonic saline solution (0.9%) preheated to 37°C. In each nostril we instilled 5 mL saline with a pipette while the subject gently flexed his/her head backwards. After

10 s, the lavage fluid was expelled and collected in plastic tubes. This procedure has shown to produce a recovery of  $7.7 \pm 1.2$  mL [16].

#### *Symptom score*

Symptoms were recorded by using a scoring system according to Lebel [24] at the time points of lavage. Symptom scores were graded in points: 3–4 sneezes = one point,  $\geq 5$  sneezes = three points, anterior rhinorrhoea = one point, posterior rhinorrhoea = one point, difficult nasal breathing = one point, one nostril blocked = two points, both nostrils blocked = three points, pruritus of the nose = one point, pruritus of palate or ear = one point, and conjunctivitis = one point (total score ranges from 0 to 11 points). In addition, the number of sneezes and the amount of secretion were noted.

#### *Mediator assays*

Lavage fluid was stored on ice and centrifuged for 10 min at 400 x *g*. The supernatant was stored at –20°C. Histamine was measured with an automated fluorometric assay [26] with a sensitivity of 0.1 ng/mL. Tryptase and ECP were determined by radioimmunoassay (RIA), according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden), with a sensitivity of 0.5 U/L and 2 µg/L respectively. Albumin was determined by automatic kinetic nephelometry with use of the Array analyser (Beckmann, Mijdrecht, the Netherlands) according to the manufacturer's instructions (sensitivity 2 mg/L).

#### *Statistical analysis*

The effect of FPANS treatment on the immediate (HDM 100, HDM 1000 and HDM 10000 BU/mL) and late (area under the curve (AUC) between 3.5 and 9.5 h) nasal reaction and on nasal hyperreactivity (AUC of histamine dose–response curve) was evaluated.

Thirteen patients received FPANS during the first treatment period and 11 during the second treatment period. For testing the carry-over effect, within patient totals over the two treatment periods were used. We considered that there was no significant carry-over effect if the means of these within patients totals did not significantly differ between the two treatment-order groups. For this test a *P*-value < 0.1 was considered significant. In case of a carry-over effect, only the data of the first treatment period were used for statistical analysis.

For testing the effect of FPANS, within patient differences over the two treatment periods were used, provided

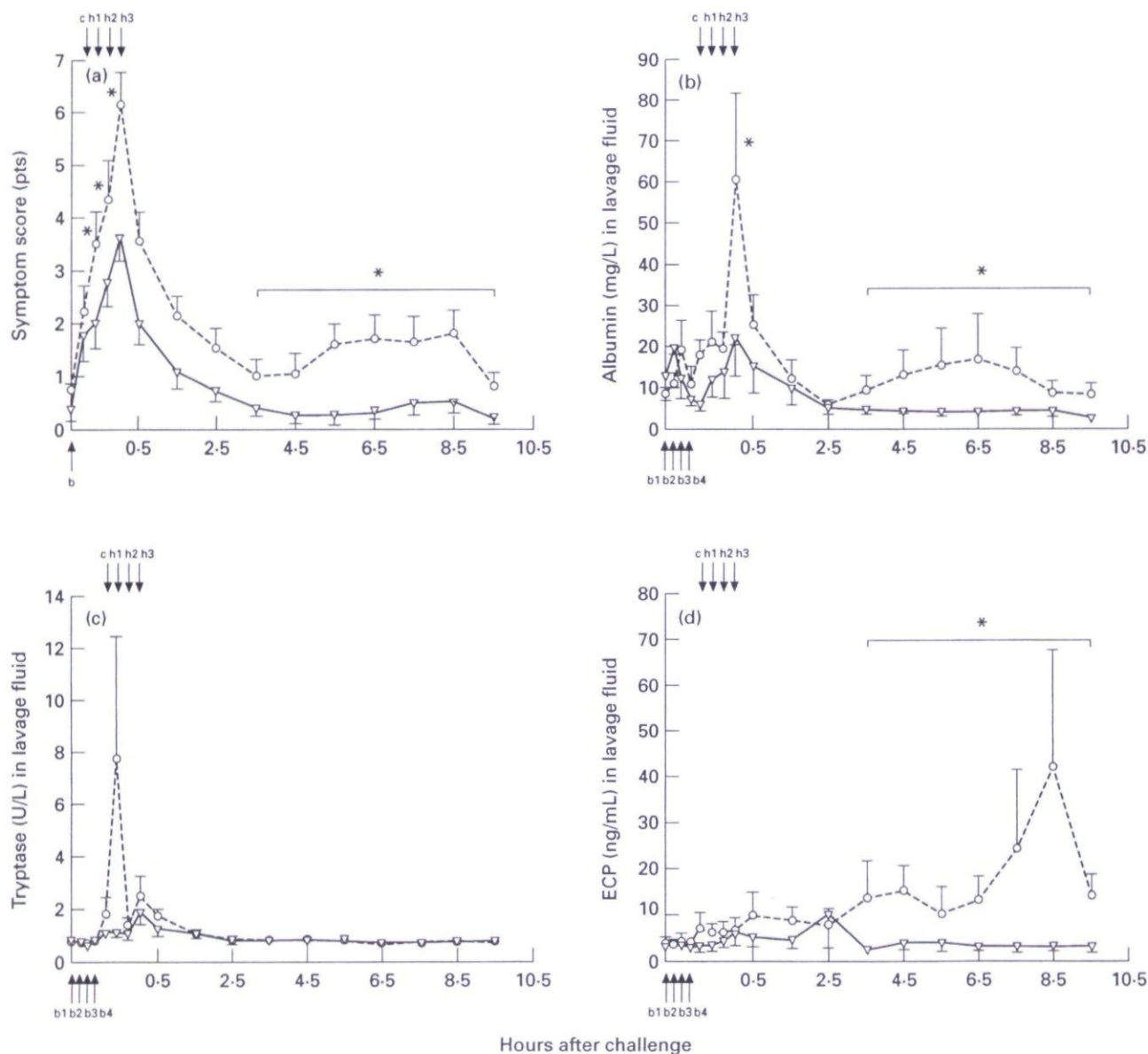


**Fig. 1.** HDM challenge; (a) Nasal response of placebo (○—○) and FPANS (▽—▽) treated groups measured for up to 9.5 h after HDM challenge. Abbreviations: (b) Baseline symptom score. b1–b4. Pre-washings. (c) 10 min after challenge with PBS. h1–h3. (d) 10 min after challenge with 100, 1000 and 10000 BU/mL HDM extract respectively. \*two-sided  $P$ -value  $\leq 0.05$ . Values are presented as mean  $\pm$  standard error of the mean (SEM).

that there was no carry-over effect. There was an effect of FPANS if the means of these within patients differences did differ significantly between the two treatment-order groups. The non-parametric Mann-Whitney  $U$ -test was used. A two-tailed  $P$ -value  $< 0.05$  was considered significant.

## Results

Figure 1 shows the results of FPANS treatment on nasal

HDM challenge. Because a carry-over effect was found for the symptom score during the immediate reaction, for this variable only the data of the first treatment period were used for statistical analysis. FPANS treatment significantly reduced the immediate symptom score with 70% ( $P=0.0104$ ), 69% ( $P=0.0045$ ) and 63% ( $P=0.0013$ ) after 100, 1000 and 10000 BU/mL of HDM extract respectively and the late-phase symptom score with 76% (AUC from 3.5 to 9.5 h,  $P=0.0003$ ). The amount of secretion was significantly diminished during

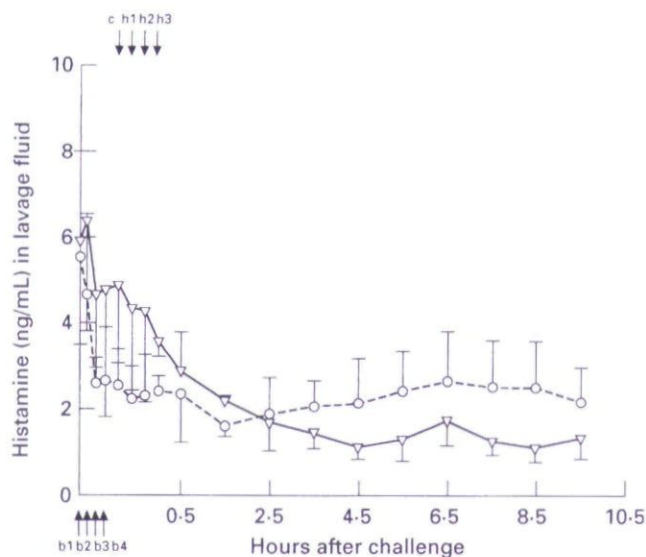


**Fig. 2.** Histamine. Histamine in lavage fluid of placebo (○---○) and FPANS (▽-----▽) treated groups in response to HDM challenge. Values are presented as mean  $\pm$  SEM.

the immediate (HDM 100 BU/mL: 32% decrease,  $P=0.0105$ ; HDM 1000 BU/mL: 49% decrease,  $P=0.0038$ ; HDM 10000 BU/mL: 17% decrease,  $P=0.0297$ ) and late response (AUC 3.5–9.5: 93% decrease,  $P=0.0464$ ) (data not shown). For the number of sneezes only an effect was observed during the early response (HDM 100 BU/mL: 57% decrease,  $P=0.0477$ ; HDM 1000 BU/mL: 77% decrease,  $P=0.0115$ ; HDM 10000 BU/mL: 53% decrease,  $P=0.0019$ ) (data not shown). Analysis of nasal lavage fluid showed a significant reduction of 62% in the amount of albumin during the early (HDM 10000 BU/mL,  $P=0.0011$ ) and of 67% during the late phase (AUC 3.5–9.5 h,  $P=0.0019$ ) after corticosteroid treatment. Tryptase release was only observed immediate after HDM challenge; treatment with FPANS tended to decrease this release (HDM 1000 BU/mL,  $P=0.0629$ ). The high mean and standard error of the mean (SEM) at HDM 100 BU/mL are mainly caused by one patient. ECP release in the period 3.5–9.5 h was almost completely inhibited (83% decrease,  $P=0.0024$ ) by FPANS.

Nasal HDM challenge did not increase histamine levels in nasal lavage fluid. No effect of corticosteroid therapy was seen (Fig. 2).

Allergen-induced nasal hyperreactivity was determined as AUC of histamine dose response curves. FPANS significantly decreased the symptom score with 34% ( $P=0.0162$ ), the number of sneezes with 41% ( $P=0.0096$ ) and the amount of secretion with 32% ( $P=0.0104$ ) (Fig. 3).

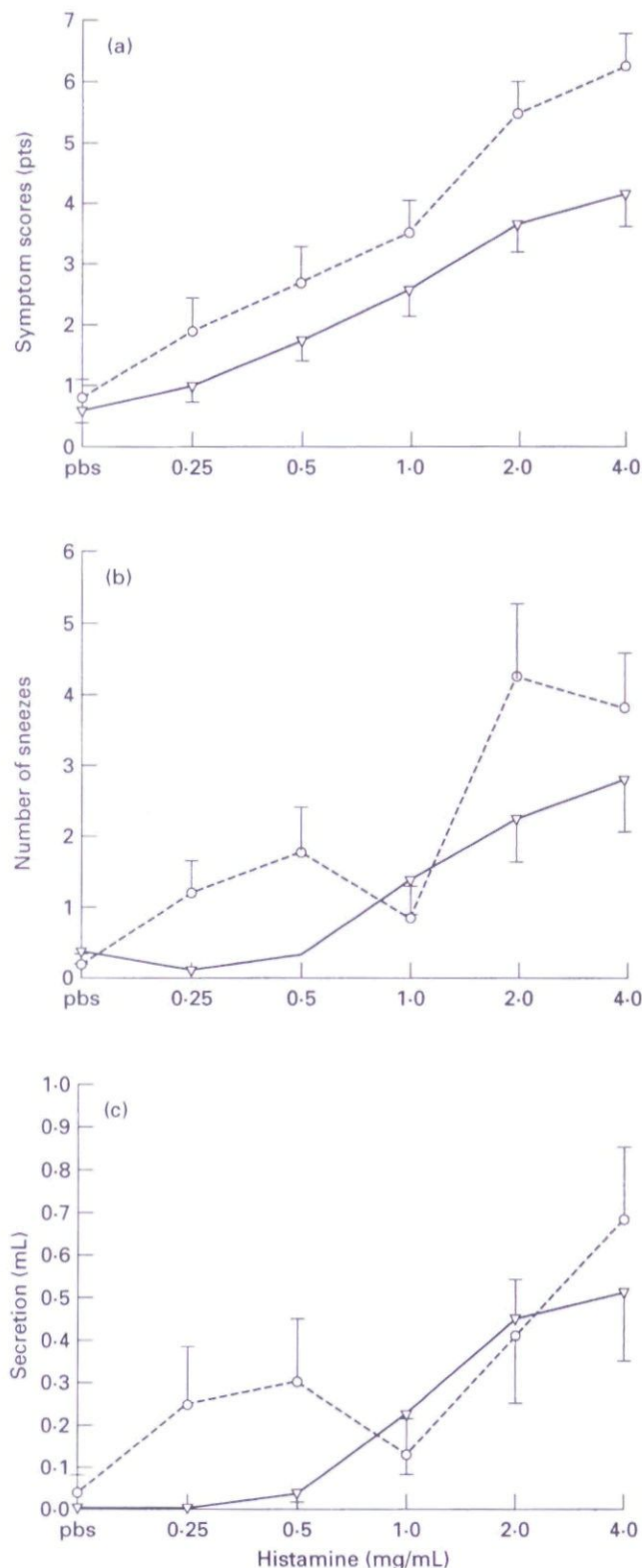


**Fig. 3.** Histamine challenge. Nasal hyperreactivity in placebo (○---○) and FPANS (▽-----▽) treated groups expressed as AUC of histamine dose response curve. Values are presented as mean  $\pm$  SEM.

## Discussion

Earlier studies mainly focused on seasonal allergic rhinitis. We studied the effect of a topical corticosteroid on the immediate and late sequelae after HDM challenge and on nasal hyperreactivity in 24 rhinitis patients allergic to HDM. Patients were challenged with high doses of HDM extract in order to obtain maximal nasal response. Since we wanted to evaluate the anti-inflammatory effects of FPANS, we were particularly interested in the effects on the late-phase response and inflammatory mediators.

Nasal symptom scores reached their maximum 10 min after challenge with HDM 10 000 BU/mL and returned to baseline levels at 3.5 h. Partial recurrence of symptoms was shown during 3.5 and 9.5 h, the period of the late-phase response [27]. However, in the nose it is difficult to define the late-phase reaction. Mygind [28] could not detect a late-phase response, clearly separated from the early response, by means of symptom scores and measurement of nasal airway resistance. In other studies late-phase reactions were determined by measurement of nasal obstruction and analysis of nasal lavage fluid [29, 30]. These studies concerned pollinosis patients.

Albumin, being the quantitative dominant protein in plasma, can be used as a marker of increased vasopermeability [31,32]. Assessment of albumin in lavage fluid demonstrated an increased influx during the immediate and the late-phase response.

To monitor the immediate response we assessed histamine and tryptase in lavage fluid. Although it is generally accepted that immediate reactions to allergen challenge are mast cell-dependent, histamine being the principal mediator, no increased release of histamine was found. This corresponds with a recent study by Gerth van Wijk [16] also in patients allergic to HDM. Linder *et al.* [33] observed a decrease of histamine in nasal secretions during the pollen season and after pollen challenge. In contrast, Naclerio *et al.* [30] measured increased histamine release after nasal challenge with ragweed pollen. Davies [34] found an increase in histamine in response to nasal grass pollen challenge, however, this release was not dose-dependent. Since the histamine levels we measured were above the detection limit of 0.1 ng/mL, our failure to detect an increased histamine release after allergen challenge is not due to insensitivity of our assay (automated fluorometric). Our failure to detect increased histamine levels may be attributed to our study design, because nasal secretion and nasal lavage fluid were collected separately, whereas others mixed secretion and lavage fluid. In addition, high levels of histamine have been detected in prechallenge lavages without association with the presence of symptoms

[35]. Measurement of histamine in nasal lavage fluid as marker of the nasal allergic response seems, due to these inconsistent observations, not ideal. In contrast, tryptase, a specific marker for mast cell activation [36], could be detected in measurable amounts during the immediate response. This finding is in agreement with the hypothesis that the immediate but not the late-phase response to allergen challenge is mast cell-dependent. A possible explanation for the discrepancy in tryptase and histamine release, might be that histamine is metabolized or removed more rapidly than tryptase, which has been demonstrated in the circulation [37]. In the present study as well as in a study performed by Rasp *et al.* [38] tryptase seems to be a useful marker of the immediate nasal reaction.

During the late-phase response activated eosinophils are involved [5]. As an indirect method to study the involvement of eosinophils, we measured ECP in lavage fluid. ECP, released from activated eosinophils was only detected during the late-phase response, suggesting that ECP can be used as a marker to detect the late-phase response.

Treatment with FPANS showed a partial but significant decrease of immediate symptoms and an almost complete inhibition of late symptoms after allergen challenge. The same was true for albumin influx in nasal lavage fluid. FPANS also reduced nasal inflammation as measured by ECP and tryptase. ECP release during the late phase was completely inhibited by topical fluticasone. This inhibition of ECP release was also shown by Lozewick [39] in 44 patients allergic to pollen. FPANS tended to decrease tryptase release. The tryptase values were widely dispersed. Probably with a larger number of patients the effect on tryptase would have reached significance. Juliusson [40] demonstrated a significant effect of FPANS on tryptase release after pollen challenge in 25 hay fever patients. Inhibition of the symptoms during immediate and late-phase response by FPANS is assumed to be due to a reduction of the number of mast cells and eosinophils, with as consequence decreased levels of mediators.

In patients with perennial allergic rhinitis, it has been demonstrated that nasal hyperreactivity is associated with inflammation [16]. In this study 2 weeks' administration of FPANS significantly inhibited nasal response to histamine. This confirms the hypothesis that inflammation is involved in nonspecific nasal hyperreactivity.

Concluding: in patients with perennial allergic rhinitis, an immediate and a late allergic response was shown by nasal symptoms as well as by inflammatory mediators in lavage fluid. Two week treatment with FPANS inhibits the immediate and in particular the late allergic response as well as allergen-induced nasal hyperreactivity. This

may occur as a consequence of a reduction of mast cells and eosinophils in the nasal mucosa as indirectly measured by tryptase and ECP in lavage fluid.

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