

Original article

Evaluation of an intranasal house dust mite provocation model as a tool in clinical research

Background: As a result of the all-year-round exposure to house dust mite (HDM), perennial rhinitis patients never have a clear symptom-free period. In this study, we investigated whether, despite these symptoms, we can still use nasal HDM provocations to study perennial allergic rhinitis and the effects of treatment.

Methods: In a parallel-group study, after 1 week treatment with either fluticasone propionate aqueous nasal spray (FPANS) or placebo, 20 patients, allergic to HDM, registered symptoms (nasal obstruction, rhinorrhoea, sneezing, pruritus and eye symptoms) using three different scoring methods [Lebel, categorical and visual analogue scale (VAS)] and peak nasal inspiratory flow (PNIF) after HDM provocations. Provocations were performed with 1000 biological units/ml and 24 h later with 10 000 biological units/ml of HDM. Before and after the provocations, nasal mucosa biopsies were taken for immunohistochemical staining to determine the number of eosinophils.

Results: House dust mite provocations resulted in an increase in symptoms and a decrease in PNIF. Even at high-dose provocation, the FPANS group registered significantly lower symptoms than the placebo group for nasal blockage, sneezing, eye symptoms, and PNIF in both early and late phases. FPANS also suppressed rhinorrhoea during the late phase and the influx of eosinophils in the lamina propria.

Conclusion: Despite the high background of symptoms, allergic responses can be induced in this perennial rhinitis model. The VAS score seems most suited to detect these changes and the suppression of symptoms by 7 days of FPANS treatment. Epithelial eosinophilia at baseline was correlated positively with the severity of the reaction after the first provocation.

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Perennial rhinitis has predominantly been studied as a naturally occurring disease (1–4). Because of the continuous all-year-round exposure to house dust mite (HDM) allergen, it is impossible to establish a clear, symptom-free baseline in perennial rhinitis patients. As a result of this constant presence of symptoms, detecting a rise in symptom scores caused by allergen provocation could be problematic.

Currently there is no defined HDM provocation model. A limited number of studies have used HDM provocations using different protocols and read-out methods (5–9). We therefore investigated the possibility

of developing such a model for HDM provocation in clinical perennial rhinitis research. For this purpose, we determined the effectiveness of different doses of HDM allergen (Der p 1) in provoking an allergic reaction in perennial allergic patients. As 'read-out' we not only used a total symptom-scoring method, but also a VAS and categorical-scoring method for measurement of rhinorrhoea, blockage and sneezing. In addition, we included two objective measures, namely peak nasal inspiratory flow (PNIF) and eosinophil count in nasal mucosal biopsies. As provocation protocols should also be able to detect treatment effects, we added an evaluation of a standard treatment for perennial allergic rhinitis [fluticasone propionate aqueous nasal spray (FPANS)]. Finally, as many challenge studies involve crossover designs, we determined the optimal washout period required to re-establish baseline values after HDM challenge.

Abbreviations: AUC, area under the curve; BM, basement membrane; BU, biological units; FPANS, fluticasone propionate aqueous nasal spray; HDM, house dust mite; NS, not significant; PNIF, peak nasal inspiratory flow; RAST, radioallergosorbent test; VAS, visual analogue scale.

Methods

Patients

Twenty-one persistent rhinitis patients (median age 42 years, range 17–63; 12 males) with positive HDM skin-prick test or radioallergen sorbent test (RAST), and having complaints for >1 year were included (Table 1). The medical ethics committee of the Erasmus MC approved of the study and all patients gave their written informed consent.

Study design

At visit 1, a baseline nasal mucosa biopsy was obtained and baseline symptom scores were recorded and after randomization, 10 patients received FPANS (200 µg in two actuations per nostril daily for 7 days) and 10 placebo. Following treatment on day 6, patients reported to the clinic for visit 2 and symptom scores and peak nasal inspiratory flow (PNIF) were measured.

After bilateral nasal provocation, using a nasal spray delivering a fixed volume of 0.089 ml at 1000 biological units (BU)/ml HDM (total amount 2 × 89 BU) (ALK-Abellø, Nieuwegein, The Netherlands) symptom scores and PNIF were recorded every 15 min. After 90 min, patients left the clinic and recorded symptoms and PNIF on an hourly basis for upto 8 h at home. After administering the study drug at home on day 7, patients re-entered the clinic for visit 3. After assessing symptom scores and PNIF and after applying local anaesthesia to the inferior turbinate, a nasal biopsy was obtained and the nasal provocation was repeated at 10 000 BU/ml HDM (total amount 2 × 890). Patients returned to the clinic on day 8, 24 h post-provocation (visit 4). Symptom scores and PNIF were assessed and a nasal biopsy was obtained. The patients were then randomized to a washout period of either 2 or 4 weeks and recorded daily symptom scores and PNIF. Upon returning to the clinic (visit 5) a nasal biopsy was obtained and a provocation with 1000 BU/ml HDM was performed. The same procedure as on visit 2 was followed. At visit 6, 24 h after provocation, a final nasal biopsy was obtained. A follow-up visit within 2 weeks concluded the study. All the visits took place between February and May 2000, outside the Dutch grass pollen season and neither vehicle provocation nor local anaesthesia led to the induction of clinical symptoms.

Assessment of symptoms

VAS. Rhinorrhoea, sneezing and nasal blockage were recorded by placing a vertical mark on a horizontal 100-mm line. Total VAS was

calculated as the sum of the three VAS scores by symptom (total range 0–300) (10).

Categorical symptoms scores. Rhinorrhoea, sneezing and nasal blockage were also recorded on a five-point scale (0, no symptoms; 1, mild; 2, moderate; 3, severe; 4, very severe symptoms), and total categorical symptom scores (0–12) were calculated by adding the three scores together.

Lebel scoring method. This total symptom score is graded in points: 3–4 sneezes, 1; ≥5 sneezes, 3, anterior rhinorrhoea, 1; posterior rhinorrhoea, 1; important anterior and posterior rhinorrhoea, 3; difficult nasal breathing, 1; one nostril blocked, 2; both nostrils blocked, 3; pruritis of the nose, 1; pruritis of palate or ear, 1; and conjunctivitis, 1 (total score ranges from 0–12) (11, 12).

Peak nasal inspiratory flow

Peak nasal inspiratory flow (in-check inspiratory flow meter with facemask; Clement Clarke, Harlow, UK) was used to measure nasal airflow as described before (13). After initial instruction and training, for each assessment, the highest value of three repeat measurements was used.

Immunohistochemistry

All biopsy specimens of nasal mucosa were taken from the inferior turbinate by the same investigator (N.B.O.) according to the method previously described (14). Eosinophil staining was performed in an alkaline phosphatase anti-alkaline phosphatase procedure using monoclonal antibody against major basic protein (BMK13) with anti-BMK13 (Sambio, Uden, The Netherlands) and numbers evaluated, as previously described (15).

Statistical analysis

For comparison of the symptom-scoring methods, Pearson’s correlation test was performed, using the baseline values. The paired Wilcoxon test was used for testing changes in symptoms caused by the provocation and the two-tailed Mann–Whitney test was used to test differences in area under the curve (AUC) between the two treatment groups after provocation. These AUCs are calculated for the first hour post-provocation (early phase) and for the remaining 23 h post-provocation (late phase). For changes in cell numbers over time, we used the Friedman and Wilcoxon test. We used the Mann–Whitney test for analysing differences between the treatment groups. *P*-values <0.05 were considered statistically significant.

Results

Of the 21 patients recruited, 19 completed the entire study programme. One patient dropped out after visit 4 because of an upper respiratory tract infection and one patient was withdrawn (after visit 4) for non-compliance with the protocol. There were no differences in patient characteristics and baseline values between the two treatment groups at the start of the study (Table 2).

Table 1. Overview of the inclusion and exclusion criteria

Inclusion	Exclusion
House dust mite allergy	Allergy to tree pollen, moulds or pets
Age between 16 and 65 years	Nasal surgery 6 months prior to the study
Non-smoking	Nasal polyps
Healthy	Significant septum deviation
Informed consent	Rhinitis caused by infection
	Immunotherapy for house dust mite
	Asthma requiring inhaled corticosteroids
	Pregnancy/lactation
	Systemic corticosteroids 2 months prior to the study
	Intranasal corticosteroids, antihistamines or other anti-allergic medication 1 month prior to the study

Table 2. Characteristics and baseline values of the patients

	FPANS	Placebo
(A) Characteristics		
Number of patients	10	10
Age	44.0 (19–62)	30.5 (17–45)
Sex (male/female)	6/4	5/5
Wheal size HDM (mm)	5 (4–9)	5 (4–20)
(B) Baseline values		
Symptoms		
Total VAS (mm)*	52.5 (13–155)	55 (6–184)
Categorical†	3 (0–7)	3 (0–6)
Lebel†	1.5 (1–7)	3 (0–7)
PNIF (l/min)	125 (60–210)	137.5 (70–260)
Eosinophils in the epithelium (per mm BM)	0.3 (0–20)	2.0 (0–10)
Eosinophils in the lamina propria (per mm BM)	3.1 (0–54)	3.4 (0–17)

Data presented as median (range).

*Total possible score = 300; †total possible score = 12.

Symptoms at baseline

At the start of the study, patients reported widely differing symptom scores. However, for a single patient, the different symptom scoring systems agreed well with each other for each separate symptom and the total scores ($P < 0.01$). In the baseline biopsies, the number of eosinophils varied considerably between patients. However, total VAS and total categorical symptom scores at baseline correlated with eosinophil numbers in the epithelium ($r = 0.63$, $P = 0.004$ and $r = 0.55$, $P = 0.14$, respectively).

Provocation with 1000 BU/ml following placebo

Using the total VAS score (Fig. 1A) for illustration, challenge with 1000 BU/ml HDM in placebo-treated patients resulted in clear early symptoms in five of 10 patients. In addition, one of these patients and one who had not experienced an early-phase response showed a clear late-phase response. In terms of individual symptoms (Fig. 1B–D), some sneezing was present during the first hour but relatively little during the remainder of the day. Where present, rhinorrhoea showed a clear early- and late-phase response, whereas nasal blockage increased immediately after provocation and tended to stay at an elevated level during the whole day.

At the 15-min post-provocation time point, both the VAS and categorical scores for rhinorrhoea showed a significant increase over the pre-challenge value ($P = 0.02$ and 0.04 , respectively) whereas the scores for sneezing, nasal blockage and the total score (VAS and categorical) did not increase significantly. Lebel, even at this time point, demonstrated a significant increase ($P = 0.02$). Seven of 10 patients reported eye symptoms and seven of 10 pruritus of nose, palate or ear during the early phase. Eye symptoms (4/10) and pruritis (6/10) were also noted by some patients during the late phase. PNIF measurements decreased significantly during the first 15 min after

provocation ($P = 0.05$), then almost normalized to baseline level, to decrease again later during the day (Fig. 2).

Compared with baseline, in both the epithelium and lamina propria of the post-provocation with 1000 BU/ml biopsies, a significant increase in eosinophil numbers was observed ($P = 0.03$ and 0.005 , respectively; Table 3). Interestingly, we found a significant correlation between the ranks of eosinophil numbers at baseline and the ranks of AUC of the symptom scores after the low-dose provocation. This correlation ($r = 0.49$ and 0.46) was present for total VAS (early and late, both $P < 0.05$), VAS nasal blockage (early and late $r = 0.58$ and 0.51 ; $P < 0.01$ and < 0.05), VAS rhinorrhoea (early $r = 0.53$; $P < 0.05$). Correlation was also found between the ranks of cell numbers at baseline and categorical symptom score for rhinorrhoea (early $r = 0.73$; $P < 0.05$) and nasal blockage ($r = 0.48$; late, $P < 0.05$). In contrast, we did not find that the symptoms correlated with the cell numbers in the 24-h biopsy after the low-dose provocation.

Symptoms after provocation with 10 000 BU/ml

Again, using total VAS for illustration, seven of 10 patients taking placebo had a clear early-phase reaction after provocation with 10 000 BU/ml. A late-phase reaction was seen in two of these patients. In contrast to 1000 BU/ml, sneezing was very prominent during the early phase but was largely absent during the late phase. Rhinorrhoea and nasal blockage showed a peak after 15 min, declined thereafter but returned during the late phase. When comparing the symptom scores, all three scoring methods showed a significant increase over pre-provocation scores in the first 15 min for all symptoms except nasal blockage. All patients in the placebo group mentioned pruritus during the early phase and seven of 10 during the late phase. Eye symptoms were reported by five of 10 patients during the early phase and by four of 10 during the late phase. Finally, although nasal blockage did not increase significantly by any of the subjective scoring methods, PNIF measurements did decrease significantly during the first 15 min after provocation ($P = 0.02$).

Twenty-four hours following the 10 000 BU/ml provocation, the number of eosinophils were increased in both the epithelium and in the lamina propria of the nasal mucosa compared with baseline ($P = 0.016$ and 0.0003 , respectively; Table 3). We did not find any correlation between the biopsies taken before or after the high-dose provocation and symptoms provoked by it.

Effect of treatment with FPANS on provocations

Since the three symptom-scoring methods were comparable in being able to detect the same changes in symptoms, we chose to only use the VAS scores for comparison between placebo and FPANS (Fig. 1). After

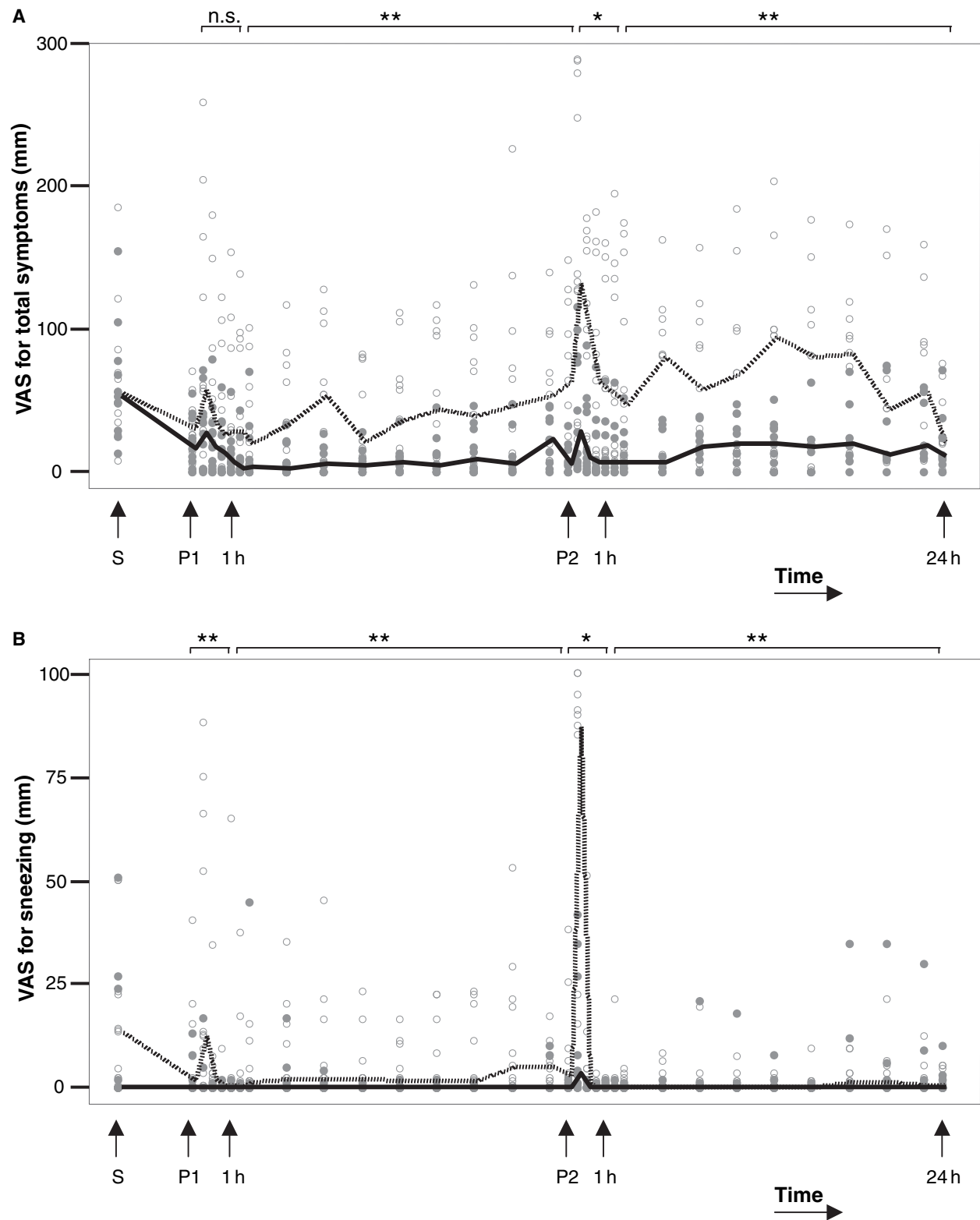


Figure 1. Symptoms after HDM provocation measured by VAS (A, total VAS; B, sneezing; C, nasal blockage; D, rhinorrhoea). Median values (line) and individual patient score (dots) are indicated (○ — FPANS and ● - - - placebo) after provocation of 1000 BU/ml (P1) or 10 000 BU/ml (P2). ** $P < 0.01$ between AUC of treatment groups; * $P < 0.05$ between AUC of treatment groups; n.s., not significant.

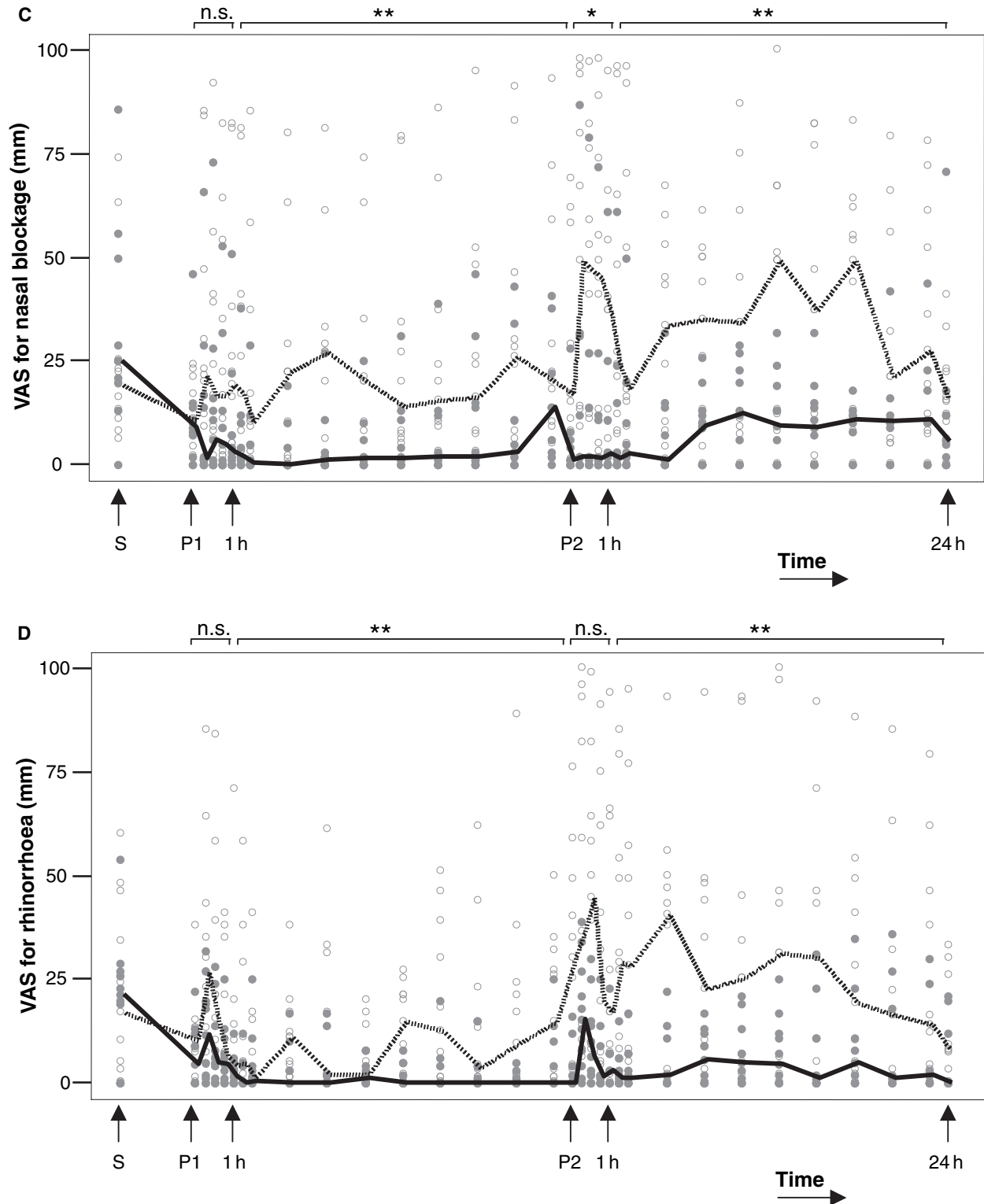


Figure 1. Continued

6 days pre-treatment with FPANS or placebo, there was no significant difference in symptom scores and PNIF between the two groups. In the FPANS group, a small

increase in total symptom score was seen immediately after the 1000 BU/ml provocation [not significant (NS) vs placebo; Fig. 1A). Only one subject showed a clear

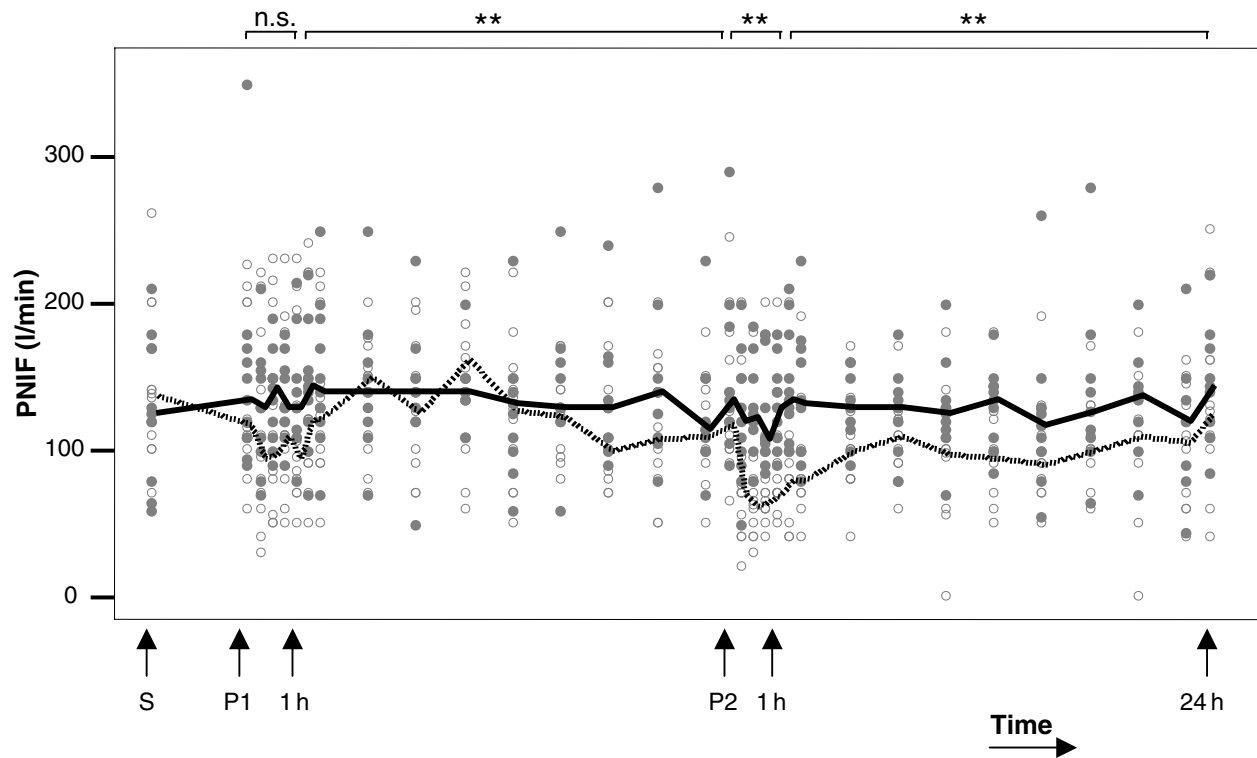


Figure 2. PNIF score after HDM provocation. Median values (line) and individual patient score (dots) are indicated (○ — FPANS and ● - - - placebo) after provocation of 1000 BU/ml (P1) or 10 000 BU/ml (P2). ***P* < 0.01 between AUC of treatment groups; n.s., not significant.

Table 3. Number of eosinophils in the nasal mucosa of both treatment groups

	Epithelium		Lamina propria	
	FPANS	Placebo	FPANS	Placebo
Baseline	0.3 (0–20.2)	2.0 (0–10)	3.1 (0–57.1)	3.4 (0–16.6)
24 h post-low-dose provocation	0.8 (0–14.0)	2.4 (0–34.8)*	9.4 (3.5–30.6)*	13.9 (0.5–65.2)**
24 h post-high-dose provocation	1.3 (0–7.9)	4.8 (0.2–15.2)*	16.2 (0–34.5)**	21.5 (0–47.4)**
Post 2 weeks washout	1.5 (0.9–7.5)	7.0 (0–22.3)	5.5 (0.9–16.9)	34.3 (6.0–42.9)**
24 h after repeated low-dose provocation	3.4 (1.4–15.2)*	12.2 (0.5–18.5)*	14.6 (2.4–41.7)*	35.3 (6.4–40.0)**
Post 4 weeks washout	1.2 (1.1–9.7)	2.3 (0–10.2)	4.4 (4.1–18.0)	14.2 (0.5–17.4)*
24 h after repeated low-dose provocation	2.1 (0.3–4.0)	1.4 (0–2.9)	8.0 (1.1–14.9)	6.9 (0.1–16.6)

Data expressed as median (range).
P* < 0.05 compared with baseline; *P* < 0.01 compared with baseline.

early-phase reaction and another a clear late-phase reaction. Total score in the late phase was significantly reduced by FPANS (Fig. 1A). Sneezing hardly occurred in the FPANS group and a significant difference between the two treatment groups during both the early (*P* = 0.004) and late phase (*P* = 0.007) was observed. Rhinorrhoea and nasal blockage increased in the first hour after provocation in the FPANS group (NS vs placebo). However, during the late phase, the FPANS group reported significantly less rhinorrhoea (*P* = 0.007) and nasal blockage than in the placebo group (*P* = 0.009)

(Fig. 1C,D). Five patients of the FPANS group reported pruritus during the first hour after provocation and four during the late phase (in the placebo group these numbers were seven and six, respectively). Interestingly, FPANS not only suppressed nasal symptoms, but also eye symptoms. These were only reported by one patient in the FPANS group during the early phase, compared with seven in the placebo group. Nobody in the FPANS group reported eye symptoms during the late phase.

After the 1000 BU/ml provocation, only a small decrease in PNIF was seen in the FPANS group in the

early phase (NS *vs* placebo), whereas during the late phase, PNIF measurement were significantly higher than in the placebo group ($P = 0.007$) (Fig. 2). In the FPANS group, the number of eosinophils was increased after provocation only in the lamina propria, when compared with baseline ($P = 0.022$).

After the provocation with 10,000 BU/ml on day 7, we found a large difference in symptoms between the two treatment groups. In the FPANS group, four of 10 patients had a clear early-phase reaction. A late-phase reaction was seen in three other patients in the FPANS group. The FPANS group showed a much smaller rise in total VAS 15 min after provocation with 10 000 BU/ml than in the placebo group ($P = 0.01$). Following a decrease within the first hour, a slight increase in total symptoms could be measured at the end of the day, although a marked difference between FPANS and placebo was still demonstrated ($P = 0.009$). After this higher provocation, compared with placebo, FPANS significantly reduced sneezing (early phase $P = 0.03$ and late phase $P = 0.009$), nasal blockage (early phase $P = 0.02$ and late phase $P = 0.009$) and rhinorrhoea (late phase $P = 0.009$) (Fig. 1C,D). During the early phase, pruritis was present in six patients and during the late phase in three patients of the FPANS group (placebo group: 10 early phase, seven late phase). Conjunctivitis was also reduced in the FPANS group; none of the patients in this group reported eye symptoms during both the early and late phase, compared with five during the early phase and four during the late phase in the placebo group. PNIF measurements were significantly higher in the FPANS group during the whole day after provocation ($P = 0.009$) (Fig. 2). Again, the number of eosinophils was increased after the 10 000 BU/ml provocation only in the lamina propria, when compared with baseline ($P = 0.004$).

Symptoms after washout

Eleven subjects had a washout of 2 weeks and nine of 4 weeks. One subject dropped out during his 4-week washout period. In the biopsies taken after either of the washout periods, the number of eosinophils in the epithelium of both treatment groups did not differ from baseline. However, in the lamina propria of the placebo group, there was a higher number of eosinophils compared with baseline both after 2 weeks ($P = 0.006$) and 4 weeks ($P = 0.009$). In the FPANS group, these numbers were comparable with baseline. After a 2-week washout period, the reaction to a 1000 BU/ml provocation was similar to that of the first provocation in patients of either treatment group. In contrast, after a washout period of 4 weeks, both groups responded with much less symptoms. In the biopsies taken after the provocation after 2 weeks washout, the number of eosinophils in the placebo group did not change compared with baseline. In the FPANS group, after the provocation, the number was

significantly higher than at baseline ($P = 0.02$). After the provocation after 4 weeks, no change from baseline was observed in either group.

Discussion

In this study, we investigated a provocation model for perennial allergic rhinitis. In line with the varied all-year-around exposure to HDM allergen in the home setting, we observed a large range of symptoms at baseline in our study group. However, despite the ongoing allergic state of the nasal mucosa, this study showed that it is possible to obtain clear provocation responses to HDM allergen in perennial allergic rhinitis patients. FPANS as a standard treatment for perennial rhinitis was able to reduce both symptoms and signs in our provocation model.

A single provocation model for HDM is preferred for studying the natural occurring disease as it is considerably more rapid and easier for controlling variables such as provocation dose (16). Some authors have combined data from pollen studies with HDM data (17, 18). However, the symptoms of seasonal allergic patients differ partly from perennial allergic patients. The first group mostly complains of sneezing, rhinorrhoea and watery eyes during the pollen season. Perennial allergic rhinitis symptoms, in contrast, predominantly consist of nasal blockage and rhinorrhoea and to a much lesser extent sneezing (19, 20). Our study reconfirmed this distinction.

The reactions to the lower provocation dose of 1000 BU/ml consisted mostly of rhinorrhoea, nasal blockage, and just a little sneezing and a treatment effect of FPANS for both the early phase and late phase could be shown. The first hour after the higher HDM provocation was more similar to the single-dose provocations described in pollen studies. Nasal blockage and rhinorrhoea were often seen during the rest of the day after provocation whilst sneezing was only observed in the first 15 min after provocation. Only some subjects mentioned eye symptoms and pruritus, something that is much more common in seasonal allergic rhinitis (19). Again, these symptoms were well prevented by FPANS. This last aspect is in accordance with the results of a meta-analysis performed by Westlund *et al.* (21) of several studies showing a positive effect of treatment with intranasal corticosteroids on eye symptoms in grass pollen-allergic patients.

We evaluated different ways of scoring symptoms in the present study. Although the results measured by categorical symptom scores were comparable to VAS, patients took more time filling them out. The Lebel scoring method, which was used by De Graaf *et al.*, is designed as a total symptom score and therefore has the disadvantage that it cannot be used to analyse individual symptoms (11, 12). Moreover, in our provocation model it seems to be less sensitive for treatment effects, because

it only showed a significant treatment effect of FPANS after the high-dose provocation. As the categorical and VAS scores hardly differed in detecting differences between treatment groups and the patients most easily use VAS scores, we would recommend the use of the VAS method. However, as patients also complain of eye symptoms and pruritis following HDM provocations, we would suggest incorporation of an additional VAS score for eye symptoms and pruritis in future studies. PNIF correlated well with the VAS score for nasal blockage and it is a little more sensitive to changes than VAS symptom score, indicating that that a small decrease in flow does not necessarily result in a subjective feeling of blockage.

In accordance with previous studies, we observed a large range in eosinophil numbers at baseline (15, 22). In contrast to earlier work by Holm *et al.* (22), we observed that the higher the steady state of perennial rhinitis

symptoms, the more the eosinophils present in the epithelium. This was also seen in seasonal allergic rhinitis (23, 24). In addition, the number of eosinophils in the epithelium would also seem to be a predictive factor of the severity of allergic rhinitis symptoms caused by provocation, according to the significant correlation found between the number of these cells at baseline and the severity of the reaction to provocation.

The optimal length of the washout periods in this model is not yet completely clear. Mostly a washout of 2 weeks is used (25, 26), but also none (27), 1 week (28), and 4 weeks (29) have been described. Although the subjective symptom scores and PNIF had returned to baseline level already after 2 weeks of washout, the number of eosinophils in the lamina propria was still higher than at baseline even after 4 weeks of washout. One might therefore conclude that a washout period of 2 weeks could be sufficient in a clinical setting.

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