

Nasal and Skin Sensitivity during Immunotherapy with Two Major Allergens 19, 25 and Partially Purified Extract of Timothy Grass Pollen

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In a double blind 3-year prospective study 40 grass pollen allergic patients were allocated to specific immunotherapy (hyposensitization) with two timothy major allergens, 19, 25, or partially purified timothy extract. The extracts were biologically standardized and coupled to aluminium hydroxide for treatment.

Efficacy of hyposensitization was evaluated by titrated nasal provocations and skin prick tests with the two treatment extracts and a five-grass mixture. The threshold dose for skin prick test (skin thresholds) produced reactions identical to histamine chloride 5.43 mmol/l, and the threshold dose for nasal provocations (nasal thresholds) produced two of the three reactions: at least 0.5 ml of nasal secretion, at least five sneezes, and/or at least a 20% fall in nasal peak flow.

Nasal thresholds showed highest efficacy from partially purified timothy extract but equal protection against timothy and the five-grass mixture. Nasal thresholds of 14 untreated patients corresponded to treatment with the two major allergens.

Changes in skin and nasal thresholds after 12 and after 64 weeks of treatment predicted the severity of hay fever. Increase in nasal thresholds coincided with a marked effect on asthma. Nasal thresholds below 1 HEP before treatment predicted major systemic side effects.

Key words: allergens; grass pollen allergy; hay fever; immunotherapy; major allergens; nasal provocation test; skin prick test.

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CLINICAL ASPECTS

Nasal and skin sensitivity were useful parameters for evaluation of clinical efficacy during hyposensitization. Changes in skin and nasal sensitivity after 12 and 64 weeks of treatment predicted severity of hay fever. Treatment with partially purified timothy extract gave better protection than the two timothy major allergens 19, 25 and even protected against four other grass species. High nasal sensitivity before treatment was associated with a high risk for systemic side effects.

Noon (25) and Freeman (17) introduced specific immunotherapy (hyposensitization), but also tissue sensitivity as a parameter of treatment efficacy using conjunctival provocations. In subsequent decades a number of researchers have shown an allergen specific reduction in the skin sensitivity during hyposensitization (3, 7, 9, 10, 16, 22, 32, 33, 35, 36) but conflicting data exist (12, 18, 23, 26). Frankland & Augus-

tin (16) were nonetheless able to show a reduced skin sensitivity from treatment with active extract but not with placebo during pre-seasonal treatment with crude as well as purified grass pollen extract, and in another placebo study with cat dandruff Taylor et al. (35) showed reduced skin sensitivity in the group treated with active cat extract but no change in the placebo group. Reduced nasal sensitivity

either qualitative (6, 7, 10, 31) or quantitative (13, 14, 15, 18, 34) and reduced bronchial sensitivity (1, 8, 24, 35) have also been shown during hyposensitization.

The present study compared hyposensitization with partially purified timothy extract and two of the timothy major allergens. Efficacy of hyposensitization was evaluated by titrated nasal provocation tests (NPT) and skin prick tests (SPT) with the two treatment extracts and a five-grass mixture. The aim of this study was to see if these parameters could monitor hyposensitization and if the treatment protected against four other grass pollen species included in the five-grass mixture.

MATERIAL

Patients

Details have been presented previously (27).

Forty typical grass pollen allergic patients were allocated to two equal groups (group WPA and group PPA) for hyposensitization with two different timothy grass pollen extracts of varying degrees of purification (extracts WPA and PPA).

Fourteen untreated grass pollen allergic patients (29) entered the trial as controls in 1979, season 2. The controls admitted for treatment just prior to the season agreed to record symptoms using the same concomitant medication as the treatment groups. After season 2 they were allocated to another trial.

Allergen extracts

The extracts were manufactured and supplied by Allergologisk Laboratorium A/S, Copenhagen, in January 1978. For SPT and NPT three extracts were used: WPA (20 antigen components from timothy grass pollen) (Alutard® SQ), PPA (two of the major timothy allergens Ag 19 and Ag 25 (20)), and FGM (a five-grass mixture composed of equal parts of orchard (*Dactylis glomerata*), yorkshire fog (*Holcus lanatus*), meadow fescue (*Festuca pratensis*), timothy

(*Phleum pratense*), and rye grass (*Lolium perenne*)). This five-grass mixture is a frequently used extract for hyposensitization. The relative quantity of Ag 25 and Ag 19 was respectively 100, 100, and 20 in the extracts WPA, PPA, and FGM, determined by quantitative immunoelectrophoresis. Aqueous and glycerinized extracts were used for NPT and SPT, respectively (27). The extracts were biologically standardized before being coupled to aluminium hydroxide for treatment. The Nordic Committee for Allergen Standardization has defined 1 HEP as 1,000 biological units (BU)/ml (2). Extracts from the same batch were used for treatment and tests.

METHODS

The experimental design is illustrated in Fig. 1. The study was double blind including the test extracts (27, 29).

Immunotherapy (hyposensitization)

Dosage has been described in detail previously (27) and the maximum tolerated dosage during perennial therapy was used as maintenance dose.

Diary cards

The score system has been published previously (27).

Symptom scores and concomitant medication were recorded daily throughout the pollen season. In the present study only total symptom scores plus concomitant medication have been used as individual parameters.

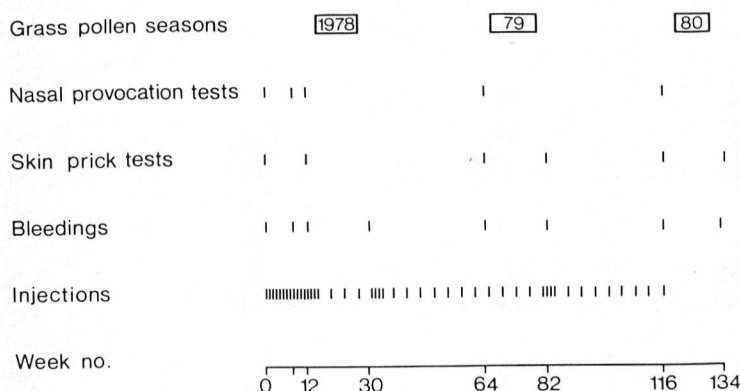
Tissue thresholds

The skin prick test (SPT) and nasal provocation test (NPT) have been described in detail previously (27).

SPT and NPT were performed with 10-fold dilutions from 0.01 to 10 HEP or until positive NPT. Both groups were tested with extracts WPA, PPA, and FGM. The controls were tested

Experimental design

Fig. 1. Experimental design.



with the same extracts but for NPT with WPA only. The threshold dose for SPT (skin thresholds) produced a reaction identical to histamine chloride 1 mg/ml (5.43 mmol/l) and for NPT (nasal thresholds) two of the three reactions: at least 0.5 ml of nasal secretion, at least five sneezes, and/or at least a 20% fall in nasal peak flow. Extract WPA of 0.2 ml 1 HEP corresponded to approximately 14,000 pollen grains (5). For SPT the wheal reactions were read when maximal for histamine after 9–12 min and for grass pollen after 13–16 min. NPT parameters were recorded 10 min after each provocation.

Statistics

Paired and unpaired Rank Sum Tests and Spearman's correlation analysis were employed using two-tailed tests with 5% significance level.

RESULTS

Symptoms and side effects

During season 1 all patients completed the trial, but one patient from each group dropped out after season 1 (29), and an additional three patients – two from group PPA and one from group WPA – refused to spare the time for nasal provocation just before season 3.

Based on evaluations from the clinical scores treatment with WPA extract was significantly better than with PPA extract during all three grass pollen seasons. The untreated grass pollen allergic patients who entered the trial in season 2, 1979, had more symptoms than the hypo-sensitized patients, but there was only a significant difference in relation to group WPA (27, 29).

Major systemic side effects (urticaria, angioedema, asthma) complicated the treatment in 33% (13/40) of the patients, equally distributed between the groups (27, 29).

Nasal thresholds

Before treatment nasal thresholds were practically identical in the two groups, as shown in Fig. 2.

During treatment the two groups deviated ($P < 0.05$) in nasal thresholds from week 64, but only for extracts WPA and FGM. Group WPA showed highest treatment efficacy with equal protection against timothy (WPA) and five-grass mixture (FGM).

Table 1 illustrates that changes in nasal thresholds up to 64 weeks of treatment for extracts WPA and FGM predicted symptoms at seasons 2 or 3. Changes in only one of the NPT parameters – sneezing, blockage, or rhinorrhoea – were insufficient for prediction of

NASAL PROVOCATION TEST (NPT)

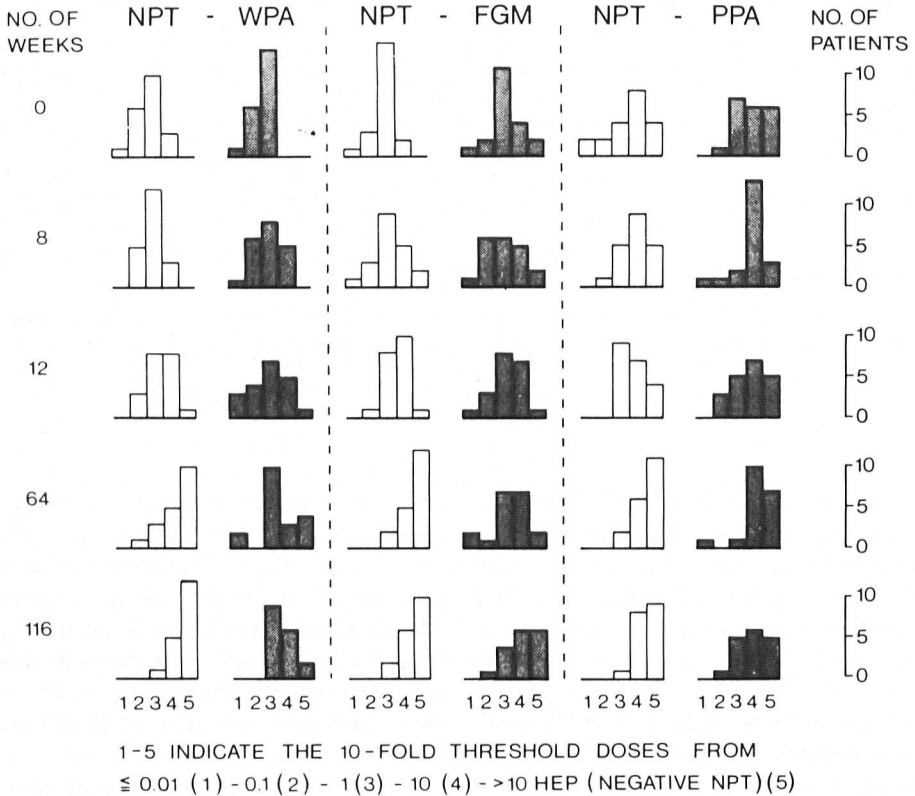


Fig. 2. Nasal thresholds for extracts WPA, PPA, and FGM in group WPA □ and group PPA ■ before (week 0) and after 8, 12, 64, and 116 weeks of treatment.

symptoms, but sneezing was the best single parameter ($r = -0.23$, $P = 0.09$)

All controls showed nasal thresholds of 10 HEP or below and were therefore comparable to group PPA at season 2.

Nasal thresholds below 1 HEP before treatment were detected in 62% (8/13) of the patients with major systemic side effects during treatment, but in only 22% (6/27) without these side effects ($P < 0.05$).

Skin thresholds

Before treatment group PPA showed somewhat lower skin thresholds than group WPA but only slightly lower, as illustrated in Fig. 3.

During treatment both groups developed higher skin thresholds already from week 12 for all test extracts but very few negative SPT and the skin thresholds did not differ between the groups.

Table 2 illustrates that changes in skin thresholds up to 12 weeks of treatment for extract WPA predicted symptoms at all three seasons. Symptom scores presented a similar picture for extracts FGM and PPA but without significance.

Skin thresholds were investigated just before and after seasons 2 and 3 (Fig. 1). The thresholds decreased during the seasons for extract FGM only, but without difference between the groups or in relation to symptoms.

Table 1

Ten-fold changes in nasal thresholds during hyposensitization vs. medians of symptom scores. Threshold changes with low *P* values are compared with symptom scores at the subsequent season. Changes in nasal thresholds up to season 2 (1979) for extracts WPA and FGM predicted symptoms at seasons 2 or 3

10-fold threshold changes	Changes in nasal thresholds vs. symptom scores (No. patients)								
	NPT-WPA			NPT-PPA			NPT-FGM		
	1978	1979	1980	1978	1979	1980	1978	1979	1980
≥ 2	126 (8)	137 (16)	22 (16)	286 (3)	315 (5)	128 (5)	175 (4)	137 (14)	32 (14)
$= 1$	248 (11)	191 (12)	99 (12)	127 (8)	129 (14)	19 (9)	134 (16)	141 (12)	12 (12)
≤ 0	148 (21)	403 (10)	58 (10)	136 (29)	188 (19)	76 (21)	193 (20)	304 (12)	163 (12)
<i>P</i>	0.33	0.04	0.19	0.54	0.80	0.13	0.53	0.07	0.04

More than 64% of the controls reacted on 1 HEP threshold before season 2 and it was not possible to prove seasonal variation or correlation to symptoms.

SPT reproducibility of double controls on the untreated patients from season 2 fulfilled the criteria from the Nordic Committee for Allergen Standardization (2) with a variation coefficient of the wheal area

$$\frac{(D + d)}{2} \text{ mm}$$

of 14% of the mean (3.4 mm) of the histamine reference 1 mg/ml (5.43 mmol/l) and 19%, 23%, and 26% of the mean (8.7–9.0–6.8 mm) with extracts FGM, WPA, and PPA respectively in concentrations of 10 HEP.

DISCUSSION

Since nasal thresholds before hyposensitization were practically identical for the two groups and then deviated during treatment, the partially purified timothy extract, WPA, gave better protection than the two major allergens, PPA. Treatment with WPA even protected against four other grasses in the five-grass mixture (FGM) which corresponds to the pronounced cross-reactions between the individual grass species (11, 21, 37).

It is notable that changes in nasal thresholds coincided with a marked effect on associated

grass pollen asthma. Clinical results (27, 29) showed treatment efficacy of extract WPA from season 1, but greater deviation in symptoms between the groups from season 2. Changes in nasal thresholds predicted symptoms from season 2. This indicates that NPT only recorded a pronounced treatment effect whereas SPT predicted symptoms already from season 1.

The dilutions of extracts for NPT were prepared each day from a 10 HEP stock solution and the change in allergen activity of the 10 HEP extracts had no essential influence on the results (30). If the nasal thresholds were influenced by seasonal variation these variables should change parallelly in the thresholds of the two groups and not alter the comparison. If no difference in nasal thresholds was shown during treatment it would have been necessary to compare with the corresponding thresholds in placebo treated patients. Our untreated patients who did not receive any placebo had nasal thresholds corresponding to treatment with PPA, which supports the results.

All patients were typical grass pollen allergics and had positive NPT to timothy (WPA), but before hyposensitization six out of 20 patients in the group treated with PPA and four out of 20 in the group treated with WPA had negative NPT for extract PPA. Prior to the study the importance of nasal reactivity for the two major allergens was not known and NPT for extract PPA was not included for diagnostic reasons but as one of the many parameters for evaluating the mechanisms during hyposensitization.

SKIN PRICK TEST (SPT)

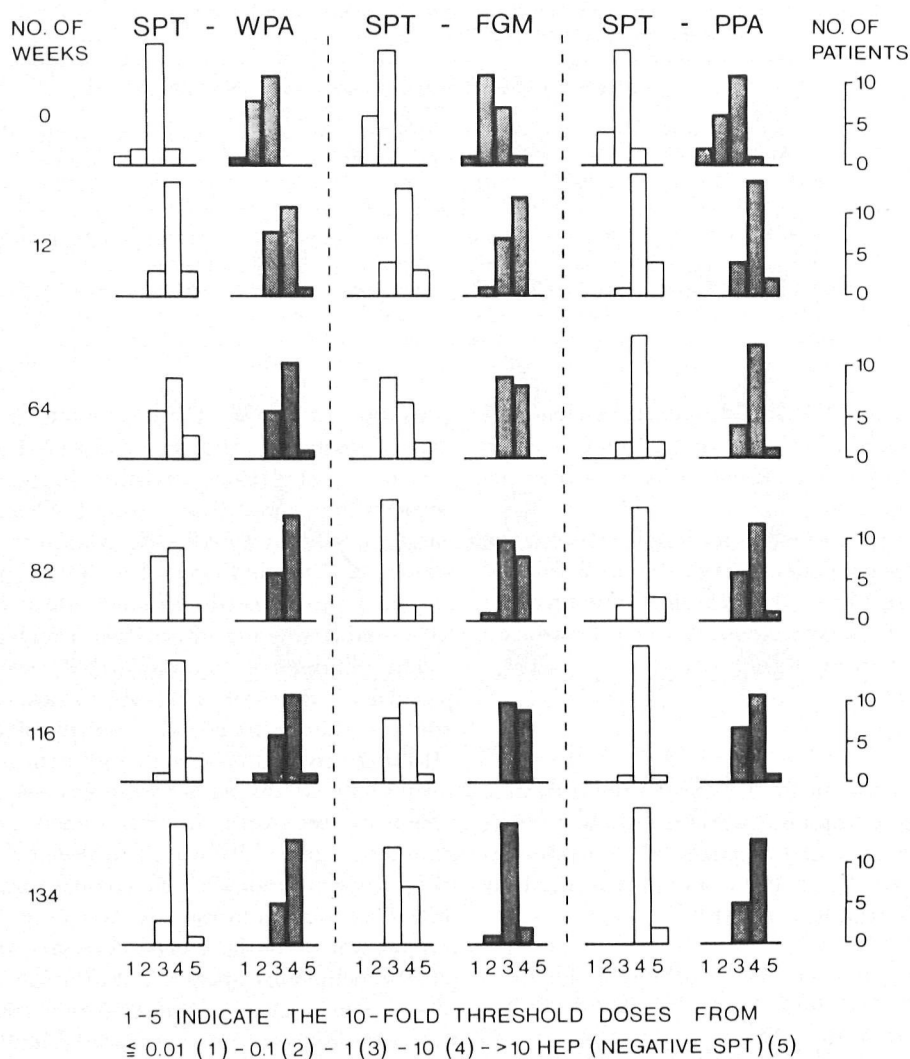


Fig. 3. Skin thresholds for extracts WPA, PPA, and FGM in group WPA □ and group PPA ■ before (week 0) and after 12, 64, 82, 116, and 134 weeks of treatment.

Three parameters were used in NPT and the nasal thresholds were defined by two of the three reactions: at least 0.5 ml of nasal secretion, at least five sneezes, and/or at least a 20% fall in nasal peak flow. Borum & Mygind (4) have evaluated the day-to-day variation for nasal provocation using the same three parameters (nasal airway resistance measured by

rhinomanometry and not by nasal peak flow). They found that sneezing gave the best reproducibility but was only acceptable (23%) when at least two of the three parameters were used as criteria for positive NPT and with at least 1 week's interval between each test. The same criteria were used in the present study.

The skin thresholds prior to hypersensitiza-

Table 2

Ten-fold changes in skin thresholds during hyposensitization vs. medians of symptom scores. Threshold changes with low *P* values are compared with symptom scores at the subsequent seasons. Changes in skin thresholds up to season 1 (1978) for extract WPA predicted symptoms at all three seasons

10-fold threshold changes	Changes in skin thresholds vs. symptom scores (no. patients)								
	SPT-WPA			SPT-PPA			SPT-FGM		
	1978	1979	1980	1978	1979	1980	1978	1979	1980
≥ 2	119 (13)	129 (11)	45 (11)	103 (13)	129 (11)	31 (8)	94 (13)	143 (8)	50 (8)
$= 1$	125 (18)	175 (18)	10 (18)	156 (22)	188 (21)	68 (24)	167 (20)	177 (21)	48 (22)
≤ 0	298 (9)	401 (9)	198 (9)	248 (5)	201 (6)	53 (6)	205 (7)	333 (9)	73 (8)
<i>P</i>	0.02	0.03	0.02	0.27	0.69	0.66	0.25	0.42	0.78

tion were less identical between the groups than nasal thresholds and this may possibly explain the lack of difference in skin thresholds between groups during treatment.

It is noteworthy that changes in skin thresholds up to 12 weeks of treatment predicted symptoms, not only at season 1 but also at the subsequent two seasons. The changes in skin thresholds until seasons 2 and 3 (from before treatment) did not predict any symptoms, but in contrast to nasal thresholds only a very few patients presented negative SPT during treatment and the majority showed skin thresholds of 1 or 10 HEP. This is consistent with the fact that the maintenance dose (maximum tolerated dosage) had to be reduced after the first season because of increase in local side effects (29). The histamine reactions in SPT did not change during treatment and the glycerinated extracts were used within approx. 4 months when no essential change in allergen activity was demonstrated (30).

In both groups most of the skin and nasal thresholds below 1 HEP before treatment switched to 10 HEP or more (negative) during treatment and patients with high changes in thresholds showed few or no symptoms. Clinical high-response in very sensitive patients was only statistically significant when evaluating by means of crossed radioimmuno-electrophoresis (28). The slightly lower skin thresholds in the group treated with PPA extract cannot prove the higher efficacy of the WPA extract as pri-

marily the patients with low skin thresholds before treatment were clinical high-responders – nor can a possible seasonal variation explain the correlation between changes in skin thresholds and symptom scores.

Patients with low tissue thresholds when untreated are assumed to have severe symptoms. Skin and nasal thresholds in the controls did not correlate to symptoms but this was attributable to the fact that the majority of the relatively few patients had thresholds of 1 HEP.

Evaluation of nasal thresholds showed equal protection against extracts WPA and FGM, but Morrow Brown et al. (7) recently described a higher efficacy from individual therapy with individual grass species as opposed to FGM extracts. Since only changes in skin thresholds for WPA extract were correlated to symptom scores and only skin thresholds for FGM decreased during seasons, as in untreated patients (19), it cannot be excluded that treatment with WPA extract protected less against FGM than WPA as a sign of specific antigens in individual grass species.

Dosage was comparable between patients with low or high thresholds but the most sensitive patients, in fact, received – relative to their sensitivity – much higher allergen stimulation than patients with low sensitivity. During perennial therapy no one, not even patients with high thresholds before treatment, tolerated a maximum dosage of 10,000 BU and therefore higher maximum dosage was not an alternative for higher efficacy.

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