

Tree-pollen allergy is efficiently treated by short-term immunotherapy (STI) with seven preseasonal injections of molecular standardized allergens

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The efficacy and tolerance of short-term immunotherapy (STI) by seven preseasonal injections of tree-pollen allergens (ALK7 Frühblühermischung®) was investigated in a double-blind, placebo-controlled, multicenter study with 111 rhinoconjunctivitis patients. Nasal and bronchial symptoms simultaneously analyzed, and nasal symptoms as a single end point, but not the overall score of nasal, bronchial, and conjunctival symptoms, showed a significantly lower increase with STI during birch-pollen exposure (both $P=0.033$, $n=105$, Mann–Whitney U-test). However, a selective analysis with patients from centers with high recruitment figures ($n \geq 10$ patients, $n=29$ STI, $n=32$ placebo) showed a significantly lower increase of nasal, bronchial, and overall symptom score (STI 11.0 vs placebo 18.0, $P=0.001$, U-test). STI had equidirected effects on conjunctival, nasal, and bronchial symptoms analyzed as multiple end points, although conjunctival symptoms were not significantly different as a single end point. The seasonal increase in drug use was reduced by 62% in the STI group compared with placebo ($P=0.032$, t -test). Specific IgG4 increased only after STI ($P<0.001$); IgE was not significantly different. Eosinophil cationic protein remained unchanged with STI, but significantly increased with placebo in the pollen season ($P=0.003$). STI was well tolerated. In conclusion, STI was shown to be efficacious and safe for the treatment of patients with tree-pollen rhinoconjunctivitis.

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Short-term immunotherapy (STI) has been shown to be clinically effective and safe in a placebo-controlled, double-blind, multicenter study with grass-pollen allergens. Grass-pollen-allergic patients developed significantly fewer symptoms and used

fewer topical nasal symptomatic drugs compared with placebo during the grass-pollen season. Skin sensitivity decreased for patients treated with the maximum dose for STI. Specific IgE and IgG4 increased after STI treatment (1). A large number

of controlled studies of specific immunotherapy have been performed with various allergens (2–7). Most placebo-controlled studies have been carried out with grass-pollen allergens, but very few with allergens from tree pollen (8). One of the reasons might be that short periods with considerable pollen exposure of the single pollen species of hazel, alder, and birch alternate with periods without symptoms for patients with rhinoconjunctivitis caused by tree pollen. This makes the analysis of symptoms for patients allergic to tree pollen more difficult than for patients allergic to other allergens with a long and continuous period of exposure.

Here we report data on the safety and efficacy of STI by seven preseasonal injections with tree-pollen allergens for the subsequent season of alder, hazel, and birch pollen from a double-blind, placebo-controlled, multicenter study performed from November 1993 to June 1994.

Material and methods

Patients

In the age range of 18–58 years, 111 patients allergic to alder, hazel, or birch pollen, as confirmed by a wheal size ≥ 5 mm in the skin prick test with birch-pollen allergens, were enrolled in the study at 14 university allergy departments. Skin prick tests with other aeroallergens were positive for beech, oak, ash, poplar, and/or willow in 89%, for grasses and/or rye in 69%, and weeds in 46% of the patients. Patients had typical rhinoconjunctivitis symptoms in the tree-pollen season from January to April (Table 1). Patients with a need for treatment of allergic asthma, perennial rhinitis, or acute infected nasal mucosa; patients on systemic glucocorticosteroids; and patients treated by specific immunotherapy in the past 3 years were excluded. Other exclusion criteria were the known contraindications of immunotherapy, according to the EAACI immunotherapy position paper (8).

The study was performed according to EC-GCP guidelines (9) and the Declaration of Helsinki (10), and was approved by the ethics committee of the Zentralklinikum, Augsburg, Germany.

Allergen extracts

The tree-allergen extracts used for STI were partially purified and standardized extracts (11) composed of equal parts of *Corylus avellana*, *Alnus glutinosa*, and *Betula verrucosa*.

Extracts for STI, skin prick test, placebo preparations, and allergens for specific IgE and IgG4 analysis were manufactured by ALK A/S, Hørsholm, Denmark.

Table 1. Clinical data of patients

	Treatment	
	STI	Placebo
Total no. of patients	51	60
Sex		
F	28	35
M	23	25
Mean (range) age (years)	34.0 (18–58)	34.9 (18–55)
Mean (range) duration of allergy (years)	10.0 (<1–36)	10.1 (<1–36)
Eye symptoms (n)		
None (0)	4	3
Mild (1)	13	19
Moderate (2)	18	25
Severe (3)	16	13
Nasal symptoms (n)		
None (0)	—	—
Mild (1)	2	5
Moderate (2)	21	25
Severe (3)	28	30
Bronchial symptoms (n)		
None (0)	16	23
Mild (1)	20	19
Moderate (2)	12	16
Severe (3)	3	2

The allergen extracts are standardized relative to an internal standard by qualitative and quantitative immunoelectrophoresis for characterization and quantitation of the major allergens, and luminescence immunoassay (LIA) inhibition for total allergenic activity, and the biologic activity of the extracts used was determined by skin prick test, as previously described (12, 13). The potency was expressed in arbitrary SE units (specific units); 1000 SE contains 1–3 μ g of tree group 1 major allergen.

For STI, a depot extract adsorbed to aluminum hydroxide (0.5% phenol) was used (ALK7 Frühblühermischung[®], ALK-SCHERAX, Hamburg, Germany); for the skin prick test, identically manufactured, partly purified extracts of the three tree species (ALK prick SQ/soluprick SQ, 10 HEP, dissolved in 50% glycerol). STI was applied from three vials with the concentrations 10, 100, and 1000 SE/ml. Placebo was the suspension buffer with ascending concentrations of histamine dihydrochloride (0.01, 0.1, and 1 μ g/ml) in order to simulate potential local reactions. The STI and placebo preparations were of identical appearance. All extracts were stored at 4°C until use; the stability assigned by the manufacturer was much longer than the total duration of the study.

STI

Patients received seven injections at weekly intervals before the expected beginning of the tree-pollen season with ascending concentrations of STI or placebo equivalent (Fig. 1). Dose modifications

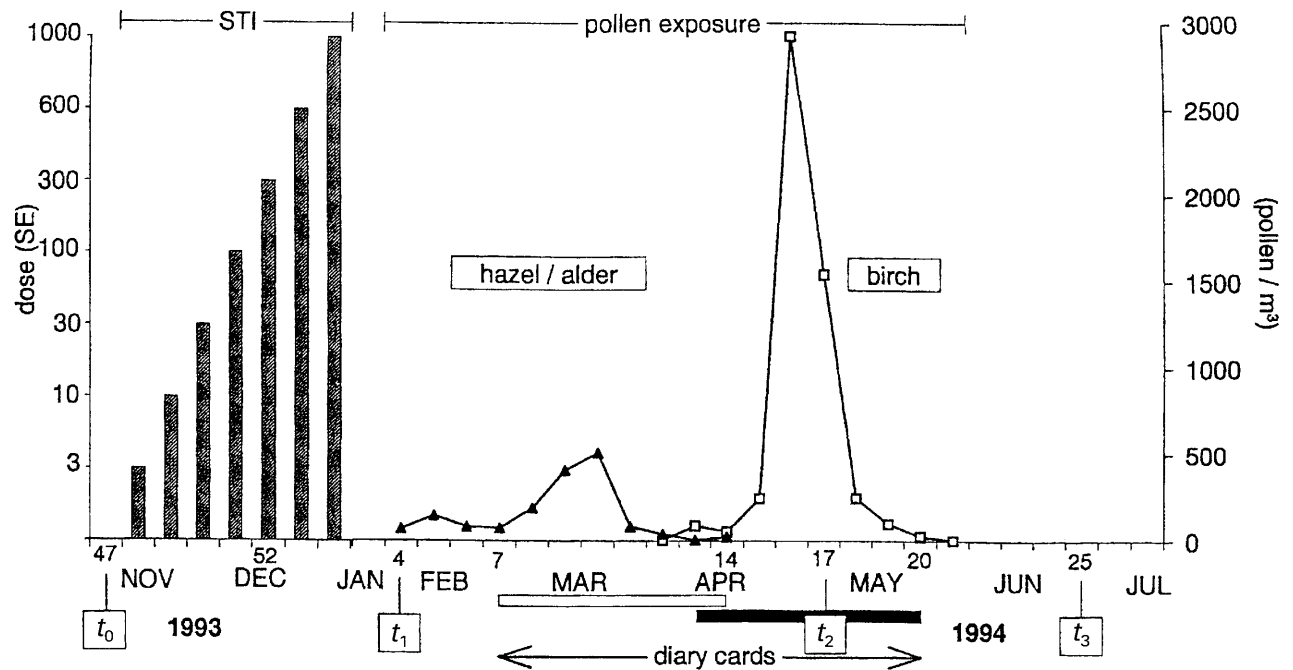


Fig. 1. Diagram of short-term immunotherapy (STI) study with tree-pollen-allergic patients, showing dosage schedule of STI and mean pollen exposure in 1994 as mean weekly pollen count for birch and as pollen count for hazel and alder pollen. Patients were enrolled in November 1993 and initially investigated before start of STI (t_0). Control investigations (t_1 – t_3) were performed after therapy (t_1), in season (t_2), and after season (t_3). Symptoms and use of symptomatic drugs were recorded by patients on diary cards during tree-pollen season from January to May 1994. STI and placebo groups were compared for period of 8 weeks in hazel/alder-pollen (white bar) and birch-pollen season (black bar), respectively.

were allowed for medical indications according to the routine procedure of specific immunotherapy (8). Patients with a modified dosage could be treated by additional injections in order to reach the maximum dose, but all injections had to be applied before tree-pollen exposure. Patients were kept under medical observation for 30 min after the injections. Disodium cromoglycate (DSCG) eye-drops and nasal sprays, local or systemic antihistamines, sympathomimetics, and local glucocorticosteroids were allowed as symptomatic treatment.

Study design

During the initial investigation (t_0), the duration and severity of symptoms of the eyes, nose, and bronchi in the pretreatment year (scored 0–3, based on memory data), general health status, and sensitization to other aeroallergens by skin prick test were recorded by the investigator, and baseline values for skin sensitivity by a titrated skin prick test and for specific antibodies were determined. Symptoms were scored and specific antibodies were determined during the three following control investigations after therapy (t_1), in season (t_2), and after season (t_3), respectively. Skin sensitivity was determined at t_1 and t_3 . Symptoms and use of symptomatic

drugs were recorded by the patient in diaries during the hazel-, alder- and birch-pollen season.

Random assignment

Patients were randomly assigned either to STI or to placebo treatment with a block size of six (three verum and three placebo preparations). According to the capacities for patient recruitment, a single block or multiple blocks were allocated to each individual trial center. Patient groups (Table 1) were comparable with respect to sex, age, duration of the allergic disease, and severity of symptoms in the previous year.

Data analysis

The main parameter was severity scores 0–3 of symptoms of the eyes, nose, and bronchi (0=no, 1=mild, 2=moderate, 3=severe symptoms) from the patient diaries. The daily symptom scores were cumulated over 1 week; therefore, the weekly score range was 0–21 score points for each individual symptom. Patient diaries were analyzed separately for the period with alder/hazel- and birch-pollen count. For statistical analysis, the week with maximum pollen count (week 0) and the weeks before (–1, –2) and after (1, 2, 3, 4) maximum pollen

Table 2. Median changes of maximum symptoms in weeks (0, 1, 2) of birch-pollen season related to reference week (-2)

Symptoms	ITT ^a (n=105)			ITT ^a (n=100)			VC ^b (n=61)		
	STI (n=49)	Placebo (n=56)	U-test	STI (n=44)	Placebo (n=56)	U-test	STI (n=29)	Placebo (n=32)	U-test
Conjunctival	5.0	4.0	0.364	5.0	4.0	0.303	4.0	6.0	0.096
Nasal	6.0	7.5	0.033	5.0	7.5	0.006	6.0	11.0	0.001
Bronchial	0.0	1.0	0.079	0.0	1.0	0.018	0.0	5.5	0.003
Overall	11.0	14.5	0.105	11.0	14.5	0.013	11.0	18.0	0.001

^a Intention-to-treat group; ^b valid-case group.

count were used. Week -2 was almost free of specific pollen exposure; therefore, it was used as reference week for the basal symptom and medication score level.

The primary study end point was determined by the mean level of total symptom scores during weeks 0, 1, and 2 of the birch-pollen season. Secondary end points were the maximum changes from week -2 baseline levels during weeks 0-2 in conjunctival, nasal, and bronchial symptoms separately, and in the medication score. The medication was scored as described previously (1).

Skin prick titration

The skin sensitivity of the patients was analyzed by skin prick test with birch allergens. The test was performed twice on both forearms with concentrations of 1, 3, and 10 HEP (histamine equivalent in skin prick test). A 0.9% NaCl solution was used as negative control, and 1% histamine dihydrochloride solution as positive control. The area of wheal and erythema caused by allergens and positive control was determined planimetrically and expressed as skin index (wheal area related to area of the positive control). The mean dose of allergen (in HEP) necessary to generate a skin index of 1 was derived from the three allergen concentrations tested.

In vitro parameters

Serum samples for *in vitro* testing were treated according to the following procedure. Complete blood was stored for 60 min (± 10 min) at room temperature and then incubated for 30 min (± 10 min) at 4°C. After centrifugation at 850 g at 4°C for 10 min, the supernatant was stored refrigerated between -20°C and -70°C. Frozen blood serum samples were freshly thawed for *in vitro* assays.

IgE and IgG4

Serum samples were analyzed for specific IgE and IgG4 by LIA with a solid phase of paramagnetic

particles (Magic Lite SQ[®], ALK), according to the instructions of the manufacturer. Results of specific IgE were expressed as standardized units per milliliter, and specific IgG4 was expressed as percentage of total activity added in the immunoassay.

Eosinophil cationic protein (ECP)

ECP in serum samples was determined by a fluorescence enzyme immunoassay (CAP/ECP-FEIA[®], Pharmacia), according to the instructions of the manufacturer.

Statistics

The primary study end point was the increase in total symptom scores during 3 weeks of high pollen exposure related to a reference week without pollen exposure. The aim was to furnish proof of statistically significant differences between STI treatment and placebo, with lower values after STI treatment ($\alpha=0.05$, one-tailed). The treatment difference was expected to be 30% of the placebo overall symptom score (standard deviation: 50%). Using a power of $1-\beta=0.80$, 36 patients per trial group had to finish the study according to protocol. The dropout rate was expected to be high due to small, inhomogeneous centers not eligible for the evaluation of efficacy. Therefore, 111 patients (STI $n=51$, placebo $n=60$) were enrolled, and 105 (STI $n=49$, placebo $n=56$) completed the trial according to protocol. A selective analysis was performed on 61 patients (STI $n=29$, placebo $n=32$) with respect to symptom and medication scores. An analysis of the multiple end points conjunctival, nasal, and bronchial symptoms was performed by the method of O'Brien (14), including the closed test procedure according to Lehman et al. (15). Data were statistically analyzed by minimum, maximum, median, arithmetic and geometric mean, standard error of mean, and frequency distribution. Baseline characteristics of treatment groups were compared by *t*-test and Mann-Whitney U-test for continuous variables, and by chi-square test for categorical

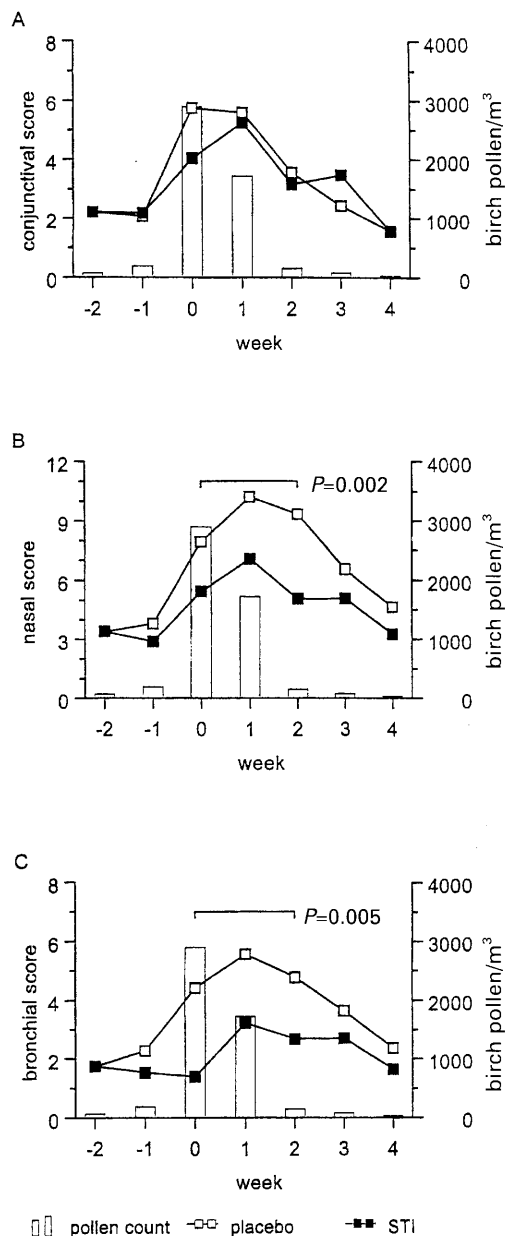


Fig. 2. Mean symptom scores and pollen count in birch-pollen season from patient diaries of conjunctival (A), nasal (B), and bronchial symptoms (C) in STI ($n=29$) and placebo ($n=32$) groups. For statistical analysis, mean levels of symptom scores for week with maximum pollen count (week 0) and weeks before (-1 , -2) and after (1 , 2 , 3 , 4) maximum pollen count were used. Data were adjusted to week -2 as reference week for basal score level.

data. Maximum changes from baseline in mean weekly symptom scores during the pollen season were compared among the trial groups by the U-test. Medication scores were analyzed by means of the t -test in a multiplicative model.

The course (t_0 – t_3) of the further outcome variables was evaluated by analysis of covariance (ANCOVA) using baseline data (t_0) as a covariate, treatment as a grouping variable, and the controls

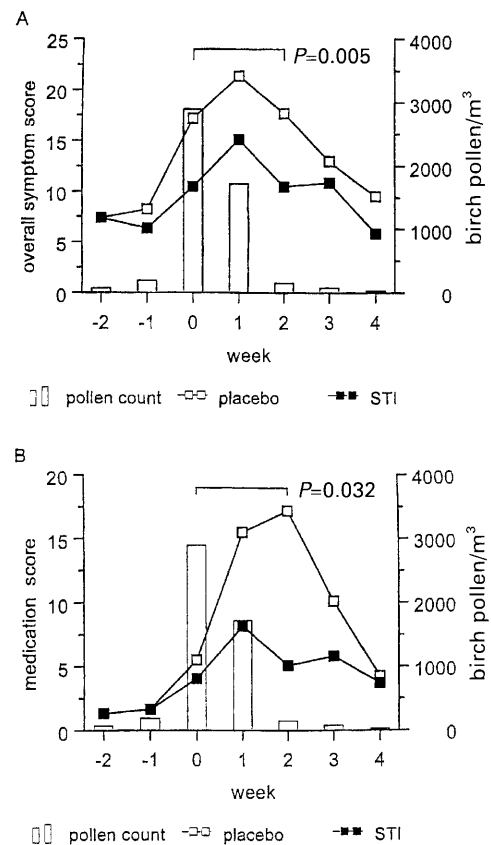


Fig. 3. Mean overall symptom scores (A) and geometric means of medication scores (B) in birch-pollen season from patient diaries in STI ($n=29$) and placebo ($n=32$) groups. Pollen count is indicated by white bars. For statistical analysis, week with maximum pollen count (week 0) and weeks before (-1 , -2) and after (1 , 2 , 3 , 4) maximum pollen count were used. Data were adjusted to week -2 as reference week for basal score level.

after therapy (t_1), in season (t_2), and after season (t_3) as a repeated measurement-dependent variable. The adjusted means at t_1 , t_2 , or t_3 , respectively, were carried out with SEM estimates from ANCOVA.

Results

Pollen exposure

Pollen counts for the period of exposure to hazel and alder pollen and for birch pollen are shown in Fig. 1. Data were available from 10 centers for hazel and alder pollen and from 13 of the total 14 centers for birch pollen. Mean maximum pollen counts were significantly higher for birch than for hazel and alder. Accordingly, the median maximum increase of overall symptoms was higher during exposure to birch pollen than to hazel and alder (14.5 for birch vs 6.0 for hazel/alder, intention-to-treat group).

Table 3. Change of nasal symptoms compared with prestudy season

Change in nasal symptoms ^a	STI		Placebo	
	n	%	n	%
Free of symptoms	12	24.5	6	10.7
Improved	33	67.3	37	66.1
Unchanged	4	8.2	10	17.9
Deteriorated	—	—	3	5.4

^a Significant differences ($P=0.038$) between STI and placebo groups by chi-square test; classifications unchanged and deteriorated were cumulated for test.

Efficacy

The general analysis of all patients with an evaluable diary (intention-to-treat group) showed a significantly lower ($P=0.041$) median maximum increase of the overall symptom score during exposure to hazel/alder pollen, with 1.5 in the STI-treated group ($n=46$) and 6.0 in the placebo group ($n=51$). During birch-pollen exposure, the median maximum increase of nasal symptoms was significantly lower ($P=0.033$) in the STI-treated group ($n=49$) than in the placebo group ($n=56$) (Table 2). It was not significant for the increase of the overall score for symptoms of the eyes, nose, and bronchi ($P=0.105$).

A selective analysis with the group of patients from centers with high recruitment figures ($n \geq 10$ patients, valid-case group: $n=29$ STI, $n=32$ placebo) resulted in significantly lower symptom scores with STI treatment for nasal symptoms, bronchial symptoms, and overall symptoms (Table 2). The mean increase in the conjunctival, nasal, and bronchial symptom scores for the valid-case group in the birch-pollen season is shown in Fig. 2A–C; the mean increase in the overall symptom score is shown in Fig. 3A.

For investigation of the contribution of conjunctival symptoms to the discrimination of the STI and placebo groups, a multiple end point analysis by the method of O'Brien (14) for the end points conjunctival, nasal, and bronchial symptoms was performed. The simultaneous analysis for the intention-to-treat group ($n=105$) was significant for nasal and bronchial symptoms ($P=0.033$), but was not significant if conjunctival symptoms were added ($P=0.105$), showing that the conjunctival symptoms did not contribute to the discrimination of the STI and placebo groups. Conjunctival symptoms for the valid-case group, however, were equidirected to the improvement of nasal and bronchial symptoms with STI treatment. The analysis of the patients not included in the valid-case group (nonvalid cases) showed a significantly higher degree of variance in symptom scores in the STI group than the placebo

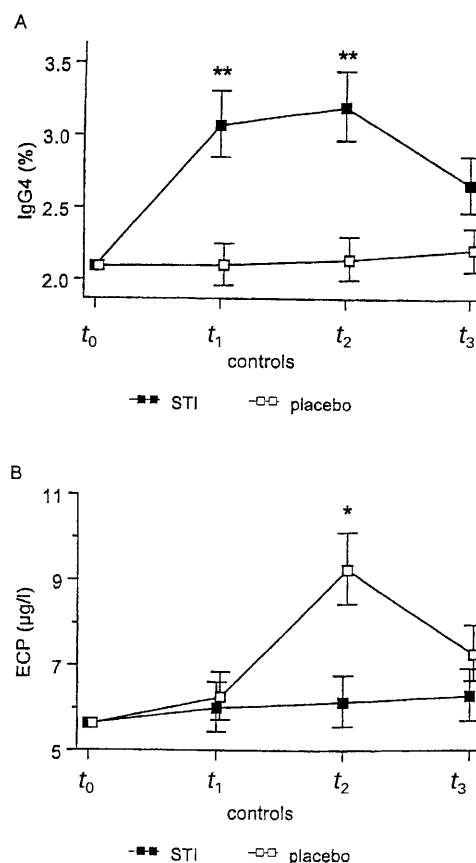


Fig. 4. (A) Specific IgG4 (relative percentage $\pm$ SEM) and (B) ECP ($\mu\text{g/l}$) as geometric means determined in patient sera (STI, $n=46$; placebo, $n=53$) at baseline (t_0), before season (t_1), in season (t_2), and after season (t_3). Values for STI and placebo were adjusted to t_0 . Statistics were performed by analysis of covariance (ANCOVA) for repeated measurements as function of time (** $P<0.001$; * $P=0.003$).

group. These high variances were caused by a few patients with extraordinarily high symptom scores. If the 10% of nonvalid cases with highest symptom scores were eliminated by a robust statistical approach, this was equivalent to the exclusion of five patients from the STI group. In the analysis of the remaining 100 patients of the intention-to-treat group, nasal symptoms ($P=0.006$) and bronchial symptoms ($P=0.018$) were significantly lower with STI treatment. The simultaneous analysis of conjunctival, nasal, and bronchial symptoms was significant ($P=0.016$), demonstrating an equidirected decrease of conjunctival symptoms together with nasal and bronchial symptoms in this group. The results for the intention-to-treat group after elimination of five patients with extraordinarily high symptom scores ($n=100$) and for the valid-case group ($n=61$) were very close (Table 2).

The maximum increase in the use of symptomatic drugs in the weeks (0, 1, and 2) of the birch-pollen season was reduced by 62% in the STI group

compared with placebo ($P=0.032$, t -test). The increase in drug use during birch-pollen exposure is shown in Fig. 3B.

Change of symptoms related to the pretreatment year

The symptoms of the patients were assessed by the investigator at all four control investigations (t_0 – t_3). The symptoms in the prestudy year (1993) were assessed by the investigator at t_0 , as were the symptoms within the study (1994) at t_2 (tree-pollen season). The change of symptoms between 1994 and 1993 could be analyzed, because treatment had been randomly given to the patients. The changes were assessed on a four-step scale (free of symptoms, improved, unchanged, and deteriorated). For nasal symptoms, significantly more patients in the STI group ($P=0.038$) became free of symptoms or improved and fewer patients were unchanged or deteriorated than in the placebo group (Table 3).

Skin sensitivity

Skin prick titration with the concentrations 1, 3, and 10 HEP was evaluated as mean dose of allergen necessary to generate the same wheal size as 1% histamine dihydrochloride ($SI=1$). No difference between the STI and placebo groups was observed at t_1 (after treatment). At t_3 (after season), skin sensitivity in the STI group was slightly, but not significantly, lower ($P=0.244$).

Specific IgE

The course of specific IgE in the STI ($n=46$) and placebo groups ($n=56$) showed only slight, insignificant differences from higher mean values after placebo. The adjusted mean courses were (t_0 – t_1 – t_2 – t_3) 61.56–67.36–61.74–68.85 SU/ml in the STI group, and 61.56–69.41–72.02–75.11 SU/ml in the placebo group.

Specific IgG4

Specific IgG4 significantly increased ($P<0.001$) in the STI group ($n=46$) but remained unchanged in the placebo group ($n=53$) at t_1 (after therapy). IgG4 continued to increase slightly in the STI group at t_2 (in season) and decreased at t_3 (after season). IgG4 only slightly increased at t_2 and t_3 in the placebo group (Fig. 4A).

ECP

ECP in serum did not change considerably in the STI group ($n=46$) during all control investigations

(t_0 – t_3). In the placebo group ($n=53$), however, ECP significantly ($P=0.003$) increased at t_2 (in season) and decreased at t_3 (after season) (Fig. 4B).

Safety

Potential allergic reactions and other adverse events were documented by the investigator after every injection on a separate data sheet.

Large local reactions as a single or multiple reaction with erythema and local swelling (>5 cm) at the injection site were documented in 10.5% of 372 total STI injections and 3.2% of 437 total injections with placebo. Systemic reactions were observed in 3.5% of the total STI injections and 8.2% of the total injections with placebo. Systemic reactions included mild to moderate symptoms of eyes and nose, mild urticaria, and swelling of the conjunctiva and eyelid. Glottis edema was reported for STI in one case after 24 h, but it was not verified by appropriate diagnostic measures and resolved without treatment. Other reactions included one mild pulse rise after 4 h and a case of shortness of breath reported as bronchospasm, which was reversible without medication. One pulse rise, one blood pressure drop, and one sickness were reported after placebo injections. No severe systemic reactions were observed.

Discussion

STI with seven preseasonal injections of tree-pollen allergens significantly reduced nasal and bronchial symptoms and, to a lesser extent, associated conjunctival symptoms.

The analysis of conjunctival symptoms in the intention-to-treat group showed results which were inconsistent with the development of nasal and bronchial symptoms. A selective analysis of the more homogeneous group of patients from centers with large numbers of recruited patients (valid-case analysis) resulted in equidirected effects of STI treatment on conjunctival, nasal, and bronchial symptoms. Of the patients excluded from the valid-case analysis, five patients in the STI group with extraordinarily high symptom scores could be identified who caused the discrepancies between the intention-to-treat and the valid-case group. Therefore, after elimination of these five patients, very similar results were obtained in the intention-to-treat and the valid-case analyses. STI-treated patients used significantly fewer symptomatic drugs than placebo patients. These findings are consistent with the results of a preceding double-blind, placebo-controlled, multicenter study with STI using grass-pollen allergens (1) and other studies for long-term immunotherapy (17, 18).

Patients were predominantly exposed to birch-pollen; exposure to pollen from hazel and alder was relatively low. The period of considerable birch-pollen exposure was 2–3 weeks long, reflecting the relevant period for analysis of patient diaries. According to the assessments of the investigators, STI-treated patients also performed better than placebo-treated patients compared to the prestudy year.

There was no significant difference between the two groups for skin sensitivity measured by skin prick testing. This might have been due to the short treatment period. For long-term treatment, an effect on skin sensitivity has been reported, at least after a minimum treatment period of 6 months (8). No significant difference between the two groups was observed for specific IgE. However, specific IgG4 significantly increased in the STI-treated patients compared with the placebo group. A preceding study with grass-pollen allergens showed that the increase of IgG4 is dependent on the dose of allergen injected in the course of STI (1). Although the increase of IgG4 cannot be correlated with the efficacy of immunotherapy, there is some evidence that IgG4 may be involved in the specific immunologic mechanism (8, 18–21).

Unexpectedly, the two groups showed significant differences in serum ECP levels. STI-treated patients had a constant level of ECP throughout the tree-pollen season, while placebo patients showed a significant increase of ECP during the pollen season. Changes of serum ECP levels in patients treated by immunotherapy have so far been reported only for patients with allergic asthma (22, 23). A significant increase in ECP levels for allergic rhinitis patients in season compared with normal controls has been reported for nasal fluid, but not for serum (24).

STI by seven preseasonal injections with tree-pollen allergens proved to be safe. No severe systemic reactions were observed in the course of 372 STI injections. Allergic reactions were restricted to a higher frequency of local reactions at the injection site and the appearance of typical rhinoconjunctivitis symptoms.

In conclusion, STI with seven preseasonal injections of tree-pollen allergens proved to be clinically effective, to reduce symptomatic drug consumption, and to influence specific immunoglobulin production and activation of inflammatory cells.

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