

Original article

Effect of tree pollen specific, subcutaneous immunotherapy on the oral allergy syndrome to apple and hazelnut

Background: The efficacy of specific immunotherapy (SIT) in pollen allergy is well established. However, its effect on pollen associated food allergy particularly the oral allergy syndrome (OAS) is not definitely ascertained.

Objective: The purpose of this controlled prospective study was to investigate whether SIT with tree pollen, mainly birch, has an effect on OAS induced by apple or hazelnut in birch pollen-allergic individuals.

Methods: Twenty-seven birch pollen-allergic subjects with OAS induced by apple or hazelnut underwent open oral provocation tests (OPT) with increasing doses (1 to 128 g) of fresh apple or ground hazelnut 1 year apart. Fifteen of 27 subjects were treated with SIT and 12 were not. Skin-prick test with birch pollen, apple and hazelnut, and specific serum IgE, IgG and IgG4 to rBet v 1, apple and hazelnut were determined.

Results: Thirteen of 15 (87%) SIT-treated subjects could eat significantly ($P < 0.001$) more of apple or hazelnut without any symptoms/signs. The average tolerated quantity increased from 12.6 to 32.6 g apple after 1 year in this group. In contrast, only one of 12 (8%) individuals without SIT was able to consume a higher amount without symptoms. On evaluating laboratory parameters, only IgG4 antibodies to rBet v 1 were found to be significantly ($P < 0.01$) increased in the SIT-treated group after 1 year.

Conclusions: The study shows that SIT with extracts containing birch pollen has a positive impact on OAS to apple or hazelnut in birch pollen-allergic individuals. In spite of this outcome, the amount of apple/hazelnut tolerated is still small. Thus, the effect of SIT on the patients' management of OAS remains limited.

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Twenty-three to 70% of individuals with pollen allergy experience enoral symptoms after eating raw fruits and vegetables (1–3). Those with a tree pollen allergy such as birch usually perceive symptoms following the ingestion of fruits or vegetables like apple, cherry, hazelnut, carrot and celery (4–6). These symptoms are referred to as the oral allergy syndrome (OAS) (5, 7, 8). The OAS reflects a phenomenon based on the cross-reactivity of specific IgE antibodies towards epitopes of certain pollen and food proteins (3, 6, 9, 10). Terms like the ragweed–melon–banana, the birch–stone–fruits–nuts, and later the mugwort–celery–carrot–spice syndromes were coined almost 30 years ago without the current knowledge of the molecular basis of IgE-mediated cross-reactivity (11, 12).

Specific immunotherapy (SIT) is an established treatment in pollen allergy (13, 14). In contrast, there is no standard treatment in food allergy including OAS except avoiding implicated food. Many patients with pollen-associated food allergy, however, ask for SIT as

therapeutic option based on the cross-reactive IgEs. Improvement of the OAS in pollen-allergic subjects using SIT has been reported to be approximately 50% (6, 15–17). Recently, Asero (18) reported a significant reduction or a complete loss of the OAS in 84% of monosensitized birch pollen-allergic subjects after SIT confirmed by an open apple challenge in a placebo-controlled prospective study. Although this reported percentage of OAS improvement appears rather high, it could suggest that SIT with pollen allergens has a beneficial effect on the OAS.

The objective of this controlled prospective study was to investigate whether birch pollen allergic subjects with an OAS induced by apple or hazelnut could profit from SIT with regard to the associated food allergy.

Methods

Patients

Of 35 randomly selected and birch pollen-allergic patients from our outpatient clinic with an OAS induced by apple or hazelnut, 27 subjects [18 females, nine males; mean age of 32 years (range

Abbreviations: OAS, oral allergy syndrome; OPT, oral provocation test; SIT, specific immunotherapy; SPT, skin prick test..

17–48 years)] agreed to participate in this study. Eight declined participation because of living in remote areas (4), lack of time (2), or no interest (2). Fifteen of 27 (56%) were treated by SIT with birch pollen extracts. Both study test periods were performed outside the pollen season. At the time of exploration, except for SIT, none of the study participants did receive any medication, nor did they experience allergic symptoms. After having signed the informed consent form, they had to complete a standardized questionnaire. Questions of interest were the course of the birch pollen allergy and the OAS symptoms over a period of 1 year in both groups of birch pollen-allergic subjects: one group treated with SIT, the other not.

Skin-prick tests

Skin-prick tests (SPT) were performed on the volar surface of the forearm with a standardized prick needle (Stallerpoint, Stallergènes, Antony Cedex, France). All study subjects were tested with reference to the recommendations of the European Academy of Allergy and Clinical Immunology with a commercially available birch pollen, apple and hazelnut extract (Soluprick, ALK, Hørsholm, Denmark) (19). In addition, fresh raw apple (Golden Delicious) and ground hazelnut were tested by the prick-to-prick technique (19). The Golden Delicious strain was chosen because of its high allergenic potency (20). Reactions were documented after 15 min by tracing the wheals with a pen, making a copy with a scotch tape and sticking it on the patients' sheet. The surface of the wheal was calculated according to the formula

$$A = \left(\frac{D_1 + D_2}{2} \right)^2,$$

where D_1 and D_2 represent the mutual perpendicular diameters of the wheal. One diameter of at least 3 mm surrounded by erythema was considered positive.

Serological tests

Serum samples were taken from each subject and stored at -20°C until used for analysis. Serum IgE, IgG and IgG4 antibodies to rBet v 1, to apple and to hazelnut were determined using the CAP FEIA system (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's instructions. Results were calculated in mg/l for IgG/G4 antibodies, and in kU/l for IgE antibodies, and expressed on a scale from class 1 to 6. IgE antibody values >0.7 kU/l (class 2) were considered positive.

Oral provocation tests

All subjects had an open oral provocation test (OPT) either with fresh apple (Golden Delicious) or ground hazelnuts. Each apple was washed, the core and stalks removed, then cut with a maximum share of skin. The patients have been given increasing amounts of apple or ground hazelnut. Before swallowing they chewed each test dose for 30 s. Oral cavity was inspected before administering each dose, which has been doubled after 5-min intervals starting with 1 g up to 128 g, corresponding to approximately two-thirds of a medium-sized apple. The OPT was stopped, when objective symptoms were recognized by the investigator, or the maximum test quantity of 128 g had been tolerated. Throughout the OPT, the study patients were under continuous observation, and any symptom was recorded. Using a hand-held peak flow meter (Labhard AG, Basel, Switzerland) peak expiratory flow was noted recording the highest of three forced exhalations before and after OPT, or in case of the occurrence of

objective signs. A significant peak flow reduction was defined as a decrease of $>20\%$ from the baseline value associated with asthma consistent symptoms. By using a visual analogue scale, each subject evaluated the intensity of any symptom perceived at each test dose. A score of 0, indicated none, a score of 10 indicated the most pronounced symptoms. OPTs were performed 1 year apart with the first test period from December 2000 to February 2001, and the second test period from December 2001 to February 2002.

Specific immunotherapy

Fifteen subjects (11 females, four males) allergic to tree pollen including birch and nine of them also allergic to grasses with a mean age of 30 years (range 17–48 years) were treated by SIT with tree pollen extract (Alutard SQ, ALK, Denmark) (13). Nine of 15 (60%) were treated with a mixed birch–hazel–alder pollen extract, and three (20%) had ash pollen added to the extract. Two individuals were treated with grass/rye pollen in combination to birch, and one patient received a 100% birch pollen extract. The patients enrolled in the study started SIT within 1.5 years. For up dosing a conventional SIT protocol with weekly injections was used followed by monthly maintenance injections (1 ml of 100 000 standard quality or equivalent units). All study participants tolerated SIT well. Twelve subjects (seven females, five males) with a mean age of 34 years (range 20–48 years) did not receive SIT and served as control group.

Statistical analysis

The Fisher's exact test was used for assessing significance in the evolution of the OAS. The Wilcoxon signed-rank test was applied for analyses of the serology and the OPT data within both groups, and the Wilcoxon rank sum (Mann–Whitney) test was applied to assess significance of the OPT between the groups. P -values <0.05 were considered significant.

Results

Ten of 15 (67%) of the SIT-treated subjects – with an average SIT duration of 18 months (range 5–36 months) – and two of 12 (17%) of the untreated group reported an improvement of the OAS after 1 year. The improvement was significantly higher in the SIT group ($P < 0.05$). All subjects receiving SIT reported improvement of the respiratory symptoms during the pollen season.

Skin-prick tests

Skin-prick tests with fresh raw apple (Golden Delicious) and ground hazelnut resulted in a higher rate of positive tests than with the commercial extracts. Skin test reactivity to apple was observed only with the native fruit. Compared with the first test period, the rate of positive SPT results to the native apple increased from 41% (11/27) to 74% (20/27) in the second test period in the SIT-treated group. Mean wheal diameters for apple and birch pollen extract did not significantly differ in both study groups (apple: $P = 0.6$; birch pollen: $P = 0.06$).

Serologic analyses

Serum IgE and IgG to apple and to rBet v 1 did not show a significant difference between the two testing periods in both study groups. However, a slight increase of the IgE to rBet v 1 was observed in the second period in both groups: from 39.1 to 42.8 kU/l in the SIT, and from 29.2 to 36.2 kU/l in the non-SIT group. While in both groups no change for IgG4 to apple was detected, there was a significant increase ($P < 0.01$; Fig. 1) of the IgG4 antibodies to rBet v 1 in the SIT-treated subjects after 1 year. The IgG4 antibody level to rBet v 1 in the serum of the SIT-treated group was significantly higher in the second period than in the group without any SIT ($P < 0.01$; Fig. 1). Because of the low number of subjects investigated, the results of IgE, IgG and IgG4 to hazelnut are not listed in detail. However, they were similar to the results with apple allergens.

Oral provocation tests

Subjective symptoms did not significantly differ between both OPTs. Recorded objective signs are depicted in Table 1. In general, objective signs occurred always later than subjective symptoms (mean: 1.8 dose-steps higher). Symptoms and signs reported in both study groups with the lowest food provocation dose of 1 g apple or ground hazelnuts are given in Table 2. Regarding objective signs, hoarseness [SIT: 13/15 (87%); non-SIT: 11/12 (92%)] and cough [SIT: 9/15 (60%); non-SIT: 5/12 (42%)] were the leading signs in both groups followed by facial erythema

Table 1. Objective signs before/after following oral provocation test with apple/hazelnut

Signs	With SIT (n = 15)	Without SIT (n = 12)	Total (%) (n = 27)
Hoarseness	13	11	24 (89)
Cough	9	5	14 (52)
Erythema of face	3	3	6 (22)
Retching	3	2	5 (19)
Swelling of lips	2	2	4 (15)
Lacrimation	0	3	3 (11)
Nose dripping	1	0	1 (4)
Ear itch	1	0	1 (4)
Mucus production	1	0	1 (4)
Reddening of palate	0	1	1 (4)

Table 2. Symptoms and signs revealed by OPT with 1 g of apple/hazelnut

	Before 1 year		After 1 year	
	With SIT (n = 15)	Without SIT (n = 12)	With SIT (n = 15)	Without SIT (n = 12)
Subjective symptoms (%)	9 (60)	10 (83)	10 (67)	11 (92)
Objective signs (%)	3 (20)	0	0	5 (42)

[SIT: 3/15 (20%); non-SIT: 3/12 (25%)]. At the second OPT 13/15 (87%) of the SIT-treated subjects tolerated a significantly higher quantity of apple ($P < 0.001$), and two did eat the same quantity of apple or hazelnut, respectively (Fig. 2). The mean quantity of apple provoking objective symptoms increased from 12.6 to 32.6 g of apple from the first to the second OPT in the SIT-treated

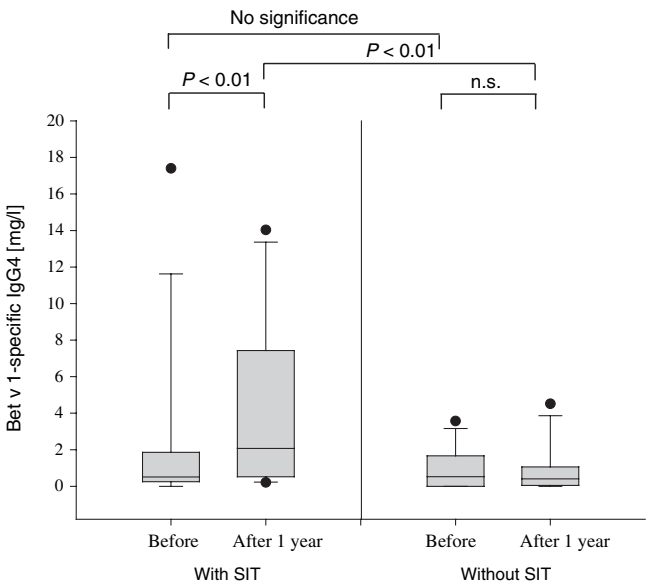


Figure 1. Bars indicate the serum levels of IgG4 antibodies to rBet v 1 (mg/l) in patients with and without SIT measured at the beginning of the study and after 1 year. (Plots representing median, the 10th, the 25th, the 75th and the 90th percentile. Circles show outliers).

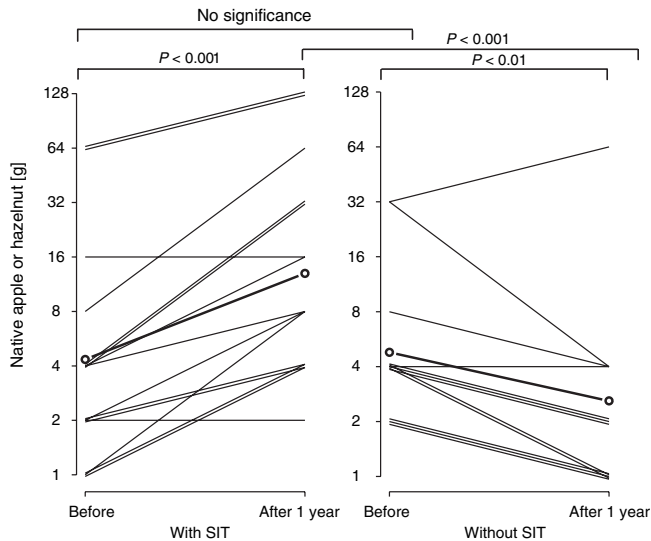


Figure 2. Oral provocation tests: food amounts provoking objective signs in patients with and without SIT before and after 1 year of treatment. Lines indicate individual trends in each subject. The bold line represents the geometric mean.

subjects. However, in subjects without SIT, the tolerated amount of apple was reduced (9.8 g at the first OPT, and 8.5 g at the second OPT) ($P < 0.01$). Only one non-SIT-treated individual could ingest a higher quantity of apple (32–64 g, respectively). A decrease of the peak flow was recorded only in one SIT-treated individual (20 and 12%, respectively). In both OPTs he noted itch of tongue and throat. No rescue medications including β_2 -agonists had to be used during and after OPT.

Discussion

In the last several years, structures, allergenic properties, and biologic functions of many protein allergens have been elucidated (2, 3, 9, 10, 21). As a result of this progress, more patients are aware of immunological cross-reactivities and ask for SIT to eliminate the annoying symptoms of OAS. However, while SIT in pollen allergic patients with rhinitis or asthma is a well established treatment, SIT is not considered as a therapeutic option in pollen-related food allergy without pollen-related symptoms even using standardized highly purified allergen extracts (13). Yet, there are some reports and studies suggesting beneficial effects of SIT in pollen-related food allergy (6, 16–18). In particular, Asero's (18) results in a prospective study showed a complete resolution of the OAS in the majority of birch pollen-allergic patients after 1 year of SIT. Our study corroborates these findings as it could be shown that over a 1-year period the SIT-treated patients tolerated higher quantities of the OAS inducing food than the non-SIT-treated group. While Asero (18) selected monosensitized birch pollen-allergic individuals for his study, we chose polysensitized patients with a clinical relevant birch pollen allergy and associated OAS as a more representative sample of our pollen-allergic patients.

The subjective awareness of improvement of OAS was confirmed by open OPT with native apple or ground hazelnuts. Although the presence of the OAS is primarily based on a subjective impression, the gold standard for diagnosing food allergy is a double-blind, placebo-controlled challenge (22, 23). Based on the established cross-reactivity of IgE antibodies to major birch pollen, apple and hazelnut allergens (9), we considered an open challenge as adequate for the study's purpose. Hence, the amount of apple or ground nuts was increased irrespective to the subjective perceptions until objective signs were noted. Hoarseness was by far the most prevalent objective symptom in both study groups followed by cough and facial erythema. Although in other investigations enoral swelling was reported to be the leading objective sign of OAS (6, 8), the symptoms recorded herein are reasonably explained as a direct consequence of an increasing local allergic reaction. The progression from OAS into hoarseness and cough might explain the occurrence of acute severe life-threatening asthma attacks in food allergic

individuals particularly in children when ignoring the OAS (24, 25).

Data on threshold values for food causing objective allergic symptoms are limited (21, 23, 26). Using double-blind, placebo-controlled food challenges Skamstrup Hansen et al. (27) found that approximately 20 g of apple was able to induce clinical symptoms in birch pollen-allergic individuals. In the SIT-treated group, the first objective signs were noticed with an average amount of 12.6 g of apple in the first, and 32.6 g in the second OPT. After 1 year of SIT, the majority of this group tolerated almost a three times higher quantity of apple. Of the non-SIT-treated group, only one individual could eat a larger quantity of apple 1 year later, which may reflect the natural evolution in pollen-related food allergy.

As expected from other studies, SIT with tree pollen extracts resulted in a significant rise of IgG4 antibodies to rBet v 1 (17, 28, 29). As the effect of SIT is largely dose-dependent, one of the possible reasons for the different outcomes in studies dealing with its results on the OAS could be that the OAS is not only because of Bet v 1 but also caused by other less well-defined cross-reactive allergens in apple, which are not present in the birch pollen extracts (30). Or the concentration of the major allergen Bet v 1 differed substantially in the commercial extracts used. Thus, the data might have been even more pronounced with extracts containing higher doses of Bet v 1.

Interestingly, no corresponding increase of IgG4 to the cross-reactive allergen in apple (Mal d 1) was observed. One may speculate that the allergen-specific immune modulation by SIT with the Bet v 1 allergen does not, or only partially, affect the immune response to the cross-reactive apple allergen. Therefore, it could be hypothesized that incorporation of cross-reactive allergens such as Mal d 1 or Cor a 1 in the SIT extract might booster the increase IgG4-antibodies and, thus, produce further clinical benefits. However, the significance of the increases of IgG4 in relation to clinical symptoms like the OAS is still not clear.

In conclusion, although this study showed a statistically significant improvement of the OAS in SIT-treated tree-pollen allergic patients as demonstrated by open OPT 1 year apart, the clinical effect for the management in patients with OAS caused by apples and hazelnuts remains limited. Thus, the severity of an OAS alone should not be considered as indication for SIT. Besides subjective symptoms, hoarseness and cough were the first objective signs of OAS in the open OPT.

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