

Original article

Randomized double-blind, placebo-controlled trial of sublingual immunotherapy with a Pru p 3 quantified peach extract

Background: Peach allergy is highly prevalent in the Mediterranean area; it is persistent and potentially severe, and therefore a prime target for immunotherapy. We aimed to study the efficacy and safety of sublingual immunotherapy (SLIT) with a peach extract quantified in mass units for Pru p 3, the peach lipid transfer protein.

Methods: Randomized, double-blind, placebo-controlled (DBPC) clinical trial. The main efficacy outcome was the change in the response to a DBPC food challenge (DBPCFC) with peach. Secondary efficacy outcomes were the changes in skin prick test (SPT), and in specific immunoglobulin E (IgE) and IgG₄ to Pru p 3. Tolerance was assessed with a careful recording of adverse events.

Results: After 6 months of SLIT, the active group tolerated a significantly higher amount of peach (three- to ninefold), presented a significant decrease (5.3 times) in SPT, and a significant increase in IgE and IgG₄ to Pru p 3. No significant changes were observed within the placebo group. Statistically significant inter-group differences were only observed in the SPT and IgG₄ responses. No serious adverse events were reported. Systemic reactions were mild, and observed with a similar frequency in both groups. Local reactions were significantly more frequent in the active group (three times) and 95% of them restricted to the oral cavity.

Conclusion: In this first exploratory clinical trial, SLIT for peach allergy seems to be a promising therapeutic option that could modify the clinical reactivity of the patients to peach intake and the underlying immunological response with a good tolerance.

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Allergy to fruits is the most frequent food allergy in adolescents and adults worldwide, and *Rosaceae* fruits are the most commonly involved (1–6). Two major patterns of allergy to this fruit family have been described. One in patients primarily sensitized to birch pollen and its major allergen Bet v 1, who subsequently develop oropharyngeal symptoms (oral allergy syndrome, OAS) caused by the presence of allergens homologous to Bet v 1 (7, 8). In areas without birch trees, *Rosaceae* fruit allergy is highly prevalent, and several studies performed in the Mediterranean area, mostly in Spain and Italy, have profiled it (7–13). Peach is involved in the majority of the patients (2, 3, 12) and seems to be the primary sensitizer (7). The clinical presentation comprises systemic reactions (SR), mainly generalized urticaria and anaphylaxis, in approximately one-third of the patients, and OAS in the remaining (7, 9). The major allergens involved in this so-called Mediterranean pattern are non-specific lipid transfer proteins (LTP), with a rate of sensitization of around

70% (7, 9, 10). Lipid transfer proteins are a family of compact and highly stable proteins that may induce sensitizations through the oral route and systemic symptoms upon oral ingestion (10, 13–15). Indeed, sensitization to Mal d 3, the apple LTP, has been shown to be a risk factor for inducing SR (7).

As for other foods, the current standard to manage peach allergy is to avoid its ingestion both fresh and processed. Peach LTP (Pru p 3) has been found in a great variety of processed foods (16), and patients can suffer potentially severe accidental reactions, and should have rescue medication including epinephrine in those at risk of anaphylaxis (7, 9, 17).

For all the reasons aforementioned, there is a need to develop specific immunotherapy to peach allergy and this trial explores for the first time the safety of such a treatment, its ability to induce immunological changes and its potential efficacy ascertained by a double-blind placebo-controlled food challenge (DBPCFC) with peach.

Methods

Trial design

The study was an exploratory randomized DBPC clinical trial performed in two centres in Spain. Patients were randomized to receive either active or placebo in a 2 : 1 proportion, respectively, during 6 months. Active treatment was a Pru p 3 quantified peach extract. A stratified block randomization was carried out to ensure a similar distribution of patients presenting with SR.

The aims of the study were to evaluate the tolerance, immunological changes and clinical efficacy. Efficacy was assessed by the change in the response to a DBPCFC with peach, performed before (T0) and after 6 months of sublingual immunotherapy (SLIT; T6). Immunological changes were evaluated by skin prick test (SPT) response and serum-specific immunoglobulin E (IgE) and IgG₄ to Pru p 3 performed before (T0), after 1 month of treatment (T1), and at the end of the trial (T6). Tolerance was assessed with a careful recording of every adverse event including nature, severity and causal relation with the treatment.

This clinical trial was approved by the corresponding ethics committees and the Spanish Drug Agency, and was conducted according to the Declaration of Helsinki, Good Clinical Practice and local regulations. All patients provided written informed consent.

Patients

The inclusion criteria were 18- to 65-year old, an immediate reaction to peach ingestion, specific IgE to peach proven by positive (≥ 3 mm) SPT either to a peach extract (ALK-Abelló, S.A Madrid, Spain) or fresh peach (prick-prick technique) and/or by a peach CAP ≥ 0.70 kU/l (Phadia, Uppsala, Sweden), and positive DBPCFC with peach. The exclusion criteria were placebo reaction in the DBPCFC with peach, previous history of food allergic reactions with hypotension, a case history of allergy to coconut, pollen immunotherapy within the previous 2 years and any clinical condition that contraindicates immunotherapy according to the European Academy of Allergy and Clinical Immunology (EAACI) guidelines (18) or that the investigators judged that inclusion might hamper the patient's safety or the study outcomes. Patients eligible for participation in the study had to meet all the inclusion and none of the exclusion criteria.

DBPCFC with peach

Double-blind placebo-controlled food challenges were performed before the start of the trial (T0) and after 6 months of SLIT (T6), in the hospital setting, out of the pollen season, following the EAACI recommendations (19). Blinded active and placebo meals were randomly administered on separate days, and prepared immediately before the challenge. In the active preparation lyophilized peach peelings were masked in an orange beverage (Sunny Delight Beverages Co., Barcelona, Spain), together with dried coconut and soluble coffee. In the placebo preparation peach was replaced by an equal amount of dried coconut. Up to seven doses were administered with a 30-min interval, and after the last dose, the patient remained under observation for at least 1 h. The starting dose contained 3 μ g of Pru p 3, and it was increased by a factor of 3 until reaching the highest dose of 2167 μ g in which case the cumulative dose of Pru p 3 given was 3249 μ g, corresponding to 200 g of pit-less unpeeled peach (16) (about 1.5 peaches of an average size). After each dose patients assessed the intensity of OAS on a 0–10 visual analogue scale (VAS). Challenges were stopped at the first objective reaction or after three consecutive doses with OAS with a

VAS score >2 . The latter criterion was included because peach-allergic patients frequently present with OAS as the sole manifestation (7, 9), and for the safety of patients, trying to avoid a potentially severe SR that might appear with higher doses of peach. The reaction was classified as local (LR, symptoms restricted to the skin or mucosal sites of direct contact with the allergen, such as OAS or isolated digestive complaints) or SR (involving organs far from the site of initial contact with the allergen, requiring absorption and dissemination, i.e. urticaria, anaphylaxis).

Peach extract

Peach extract was obtained from fresh peelings and quantified in micrograms of Pru p 3 as previously described (16). The SLIT and SPT extracts were prepared as a glycerinated, phenolated saline solution of peach extract. The placebo preparation was a similar solution without allergen.

Immunotherapy schedule

The treatment was administered sublingually (sublingual-swallow technique) and comprised four vials containing 0.4, 2, 10 and 50 μ g/ml of Pru p 3 or placebo. The schedule is shown in Table 1. Diary cards were handed out to the patients for the recording of any adverse event. During the maintenance phase, the patients visited the clinics once a month and handed back the diary cards and SLIT vials for monitoring safety and compliance.

Skin prick tests

Skin prick tests were performed at T0, T1 and T6 with four peach extracts containing 0.4, 2, 10 and 50 μ g/ml of Pru p 3, and with 10 mg/ml histamine and saline, as positive and negative controls respectively. Skin prick tests were performed in duplicate on the volar surface of the forearm by the same investigator throughout the study, following the EAACI recommendations (20). Skin prick tests were recorded at 15 min, and the wheal areas were measured by planimetry.

In vitro parameters: specific IgE and IgG₄ to Pru p 3

Serum samples were collected at T0, T1 and T6, and processed together at ALK-Abelló S.A. in Madrid (Spain) at the end of the study by an investigator blinded to the treatment. Specific IgE to

Table 1. Immunotherapy schedule

Day	No. doses	Total daily dose of Pru p 3 (μ g)
Rush build-up phase (hospital)		
1	3	0.22
2	3	1.12
3	3	5.60
4	3	28.0
5	1	50.0
1st maintenance week (hospital)		
Monday–Friday	1/day	10.0
Home maintenance (6 months)		
Monday	1	10.0
Wednesday	1	10.0
Friday	1	10.0

rPru p 3 was determined by the ADVIA Centaur® (Bayer Health-Care Diagnostics Division) platform (11, 21) and specific IgG₄ to nPru p 3 by means of ELISA. rPru p 3 was produced in *Pichia pastoris*, (22) and had an equivalent immunological reactivity to the nPru p 3 (23) purified from peach peels (24). The purity of the natural and recombinant Pru p 3 was checked with sodium dodecylsulphate-polyacrylamide gel electrophoresis and matrix-assisted laser desorption/ionization mass spectrometry analysis (23, 24).

Statistical analysis

Descriptive statistics included frequencies for qualitative variables, and mean value and standard deviation for quantitative variables, except for specific Ig and SPT results in which geometric means with their 95% confident intervals (CI) were calculated. The responses in the DBPCFC were studied by survival analysis (Log Rank test), with the challenge dose as time and a positive reaction (LR or SR) as event. This test was applied to study intra-group differences between T0 and T6, and for inter-group differences at T6. In addition, intra-group differences were analysed by the Wilcoxon test, and inter-group differences by a discrete-time survival analysis, in which the study-end score was the outcome variable and baseline score and therapy were the regressors (25). Changes in SPTs were studied by parallel-line assay and were expressed as the cutaneous tolerance index (CTI), which is the ratio of the allergen extract concentrations provoking the same skin response (26). Serum IgE and IgG₄ values were analysed by ANOVA for repeated measures. The frequencies of adverse reactions between active and placebo groups were compared with chi-squared test or Fisher's exact test. Two-sided tests were used, and *P*-values < 0.05 were considered significant. Statistical analysis was performed with SPSS (SPSS Inc., Chicago, IL, USA) and SAS software (SAS Institute Inc., Cary, CN, USA).

Results

Out of 74 patients screened, 37 were randomized in the active group and 19 in the placebo group. Four patients withdrew from the active group: two for unknown reasons, one because of personal reasons and one for allergic symptoms not related to the treatment. In the placebo group, two patients withdrew for personal reasons, and one was lost to follow-up (Fig. 1). No differences were found in the demographic and clinical characteristics of the randomized patients at T0 between active and placebo arms (Table 2). The trial was completed by 49 patients, 33 in the active group and 16 in the placebo group. Only one subject could not reach the maintenance dose of 10 µg of Pru p 3, and maintenance therapy was carried out with 2 µg. Efficacy was analysed with the 49 patients who completed the trial, whereas all the patients that received at least one dose of immunotherapy were included in the tolerance assessment.

Efficacy

DBPCFC with peach. No significant changes were observed within the placebo group (Log Rank test, *P* > 0.05 for LR and SR). Median, inter-quartile range (IQR) and Wilcoxon *P*-values for the change in the dose

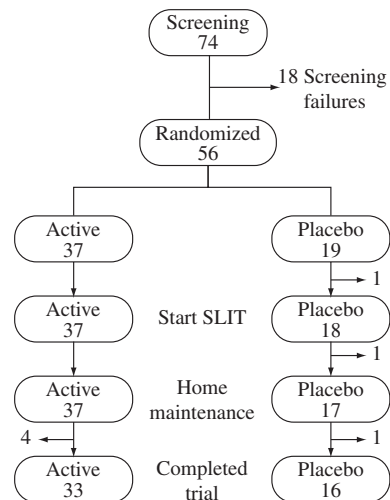


Figure 1. Flow diagram of patients.

provoking a LR outcome were 1 (−0.5 to 3) (*P* = 0.16), and 0 (−1 to 2) (*P* = 0.48) for SR outcomes (Fig. 2A). In contrast, significant changes were found in the active group (Log Rank test, *P* = 0.005 for both LR and SR) with median and IQR changes of 2 (0–3) (*P* = 0.004) for LR, and 1 (0, 2) (*P* = 0.05) for SR (Fig. 2A). This means that after 6 months of SLIT in the actively treated patients the dose of Pru p 3 needed to induce LR and SR were 9 (3²) and 3 (3¹) times higher respectively. Inter-group differences at T6 for SR were almost significant (Log Rank test, *P* = 0.06) (Fig. 2B). However, the discrete-time survival analysis performed to analyse differences between active and placebo groups adjusted for the baseline score did not reach the significance level (Fig. 2B). The negative sign of β means that hazard decreases, i.e. survival (oral tolerance in the DBPCFC) increases. The hazard ratios for LR and SR were 0.984 and 0.498, respectively, translating in a relative reduction rate (1 – hazard ratio × 100) of 2% for LR and 50.2% for SR.

The great majority of subjects who completed the trial were sensitized to Pru p 3. Positive SPT to the 50 µg Pru p 3 extract were detected in 29/33 patients in the active group (87.9%) and in 15/16 subjects in the placebo group (93.8%). The respective figures for positive (≥ 0.35 kU/l) IgE to rPru p 3 were 81.8% and 87.5%. No significant differences in efficacy assessed by DBPCFC were found between patients sensitized or not to Pru p 3, and therefore they were analysed together as described in the paragraph above.

SPT response to peach extract and specific IgE and IgG₄ to Pru p 3. The patients in the active group had a significant decrease in SPT to the Pru p 3 quantified peach extract. The CTI was 1.83 (95% CI: 1.16–2.87, *P* < 0.05), meaning that after 6 months of SLIT, a concentration of peach extract 1.83 times higher was needed to induce the same wheal area as in T0. Patients in the placebo group did not show

Table 2. Demographic and clinical characteristics of the randomized patients at T0

	Active (<i>n</i> = 37)	Placebo (<i>n</i> = 19)
Age, years [mean (SD)]	29.1 (6.1)	29.7 (7.8)
Gender (F/M)	22 (59.4%)/15 (40.6%)	10 (52.6%)/ 9 (47.4%)
Peach allergy duration*, years [mean (SD)]	13.9 (7.5)	14.6 (8.3)
Peach symptoms (%)		
Only local (OAS)	23 (62.2)	12 (63.2)
Only systemic	5 (13.5)	3 (15.8)
Local and systemic	9 (24.3)	4 (21.0)
Associated pollen allergy	25 (67.6)	10 (52.6)
Symptoms of pollen allergy (%)		
Rhinitis	2 (8)	1 (10)
Rhinoconjunctivitis	13 (52)	6 (60)
Rhinoconjunctivitis and asthma	8 (32)	3 (30)
Missing data	2 (8)	—
Other food allergies (%)	36 (97.3)	19 (100)
SPT to peach extract (mm ²)†	79.3 (62.2–101.2)	71.9 (50.3–102.8)
Specific IgE to rPru p 3, kU/l [gm (95% CI)]	3.07 (1.73–5.47)	3.42 (1.54–7.56)
IgG ₄ to nPru p 3, AU/l [gm (95% CI)]	0.106 (0.06–0.18)	0.1 (0.05–0.21)
Frequency of positive IgE (≥0.35 kU/l) to rPru p 3	78.4% (63.8–92.9%)	78.9% (54.4–93.9%)
DBPCFC with peach		
Subjective LOAEL	3 µg Pru p 3 (oral itching)	9 µg Pru p 3 (oral itching)
Objective LOAEL	9 µg Pru p 3 (erythema)	9 µg Pru p 3 (rhinoconjunctivitis)

SD, standard deviation; F, female; M, male; gm, geometric mean; CI, confidence interval; LOAEL, lowest observed adverse effect level.

No significant differences ($P > 0.05$) between active and placebo groups were observed.

*From first reported reaction to T0.

†At 50 µg/ml Pru p 3 dose (three patients from the active and one from the placebo groups were negative, but had positive prick–prick tests with fresh peach).

changes in the SPT response (CTI 0.87, 95% CI: 0.48–1.55, $P > 0.05$). Significant differences were observed between the study arms at T1 (CTI 0.39, 95% CI: 0.15–0.92, $P < 0.05$) and at T6 (CTI 0.19, 95% CI: 0.07–0.47, $P < 0.05$), translating in a lower skin reactivity in the active group of 2.6 and 5.3 times at T1 and T6 respectively.

Specific IgE to rPru p 3 showed a significant increase both in the active ($P < 0.001$) and placebo ($P = 0.025$) groups, although the increase remained only significant at T6 in the former (active 4.23, $P < 0.001$; placebo 4.04, $P = 0.079$, *T*-test). However, no significant inter-group differences were observed ($P = 0.456$) (Table 3). None of the patients with negative IgE to rPru p 3 at T0 converted to positive at T1 or T6.

IgG₄ to nPru p 3 showed a different evolution between groups ($P = 0.022$) with a significant increase in the active arm ($P = 0.007$) not observed in the placebo one ($P = 0.185$) (Table 3).

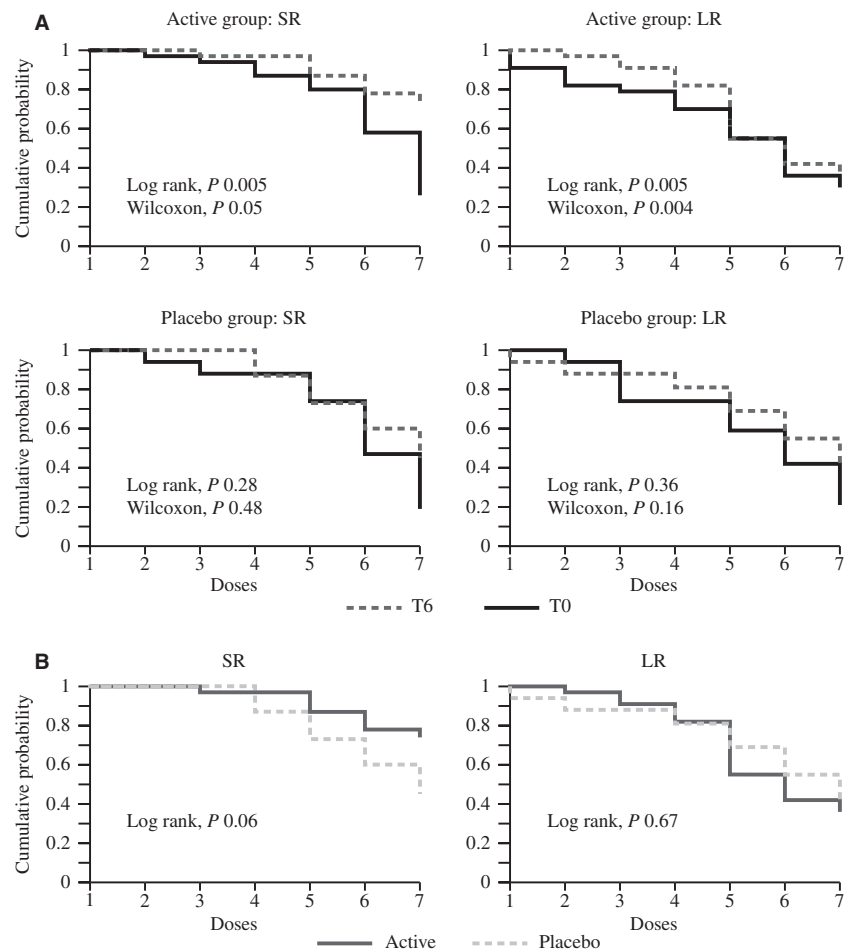
Safety

A summary of doses and adverse reactions is given in Table 4. From a total of 1480 adverse events recorded, 1356 were assessed by the investigators as probably and/or possibly related to the treatment and thus considered as adverse reactions: 1344 in the active group, and 12 in the placebo group ($P < 0.0001$). No serious adverse events were reported during the trial.

In the active group 98.8% ($n = 1328$) of the adverse reactions were classified as LR, 94.9% of them

($n = 1260$) were located on the oropharynx, and the remaining ones consisted on transient gastric complaints. Local reactions were more frequently recorded during the build-up phase and the first maintenance week ($P = 0.014$) whereas during the home maintenance up to 40.5% of patients did not present any type of complaints. Systemic reactions were recorded on 16 occasions, 14 of them during the build-up phase, one (rhinoconjunctivitis) during the hospital maintenance week and one (gastrointestinal complaints) during the home maintenance. The SRs of the build-up phase consisted on skin reactions in six patients (itching in four, erythema in two and one mild urticaria), rhinoconjunctivitis in one subject, and digestive symptoms in seven individuals (gastric complaints in one, cramps in three, diarrhoea in three). All the SRs were of mild intensity and subsided either spontaneously or with oral antihistamines, antacids and/or omeprazol. Systemic reactions were neither associated ($P > 0.05$) with the amount of Pru p 3 administered nor with a previous history of SRs with peach. No differences ($P = 0.80$) were found in the titres of IgE to rPru p 3 between patients who experienced SRs and those who only had LRs.

In the placebo group, three SRs were observed, one in the build-up phase (cutaneous itching), and two in the first maintenance week (one angioedema and one diarrhoea). When compared with the active group (Table 4), there was no difference in the frequency of SRs ($P > 0.05$), but the number of LRs was significantly lower ($P < 0.0001$).



	Discrete time survival analysis					
	Local reactions			Systemic reactions		
	β	SE	P	β	SE	P
Baseline score	-0.063	0.100	0.53	0.182	0.188	0.33
Active therapy	-0.016	0.472	0.97	-0.697	0.558	0.21

SR: systemic reaction; LR: local reaction

Figure 2. Efficacy analysis: results of DBPCFC with peach before (T0) and after 6 months (T6) of SLIT.

Table 3. Results of the IgE and IgG₄ responses to Pru p 3

	Active				Placebo				Inter-group P -value†
	T0 ($n = 37$)	T1 ($n = 37$)	T6 ($n = 33$)	P -value*	T0 ($n = 17$)	T1 ($n = 17$)	T6 ($n = 16$)	P -value*	
IgE rPru p 3	3.08 (1.73–5.48)	4.94 (2.63–9.28)	4.23 (2.17–8.23)	0.001	3.42 (1.55–7.57)	4.78 (2.00–11.4)	4.04 (1.61–10.13)	0.025	0.456
IgG ₄ nPru p 3	0.11 (0.06–0.18)	0.12 (0.07–0.20)	0.13 (0.07–0.24)	0.007	0.10 (0.05–0.22)	0.10 (0.05–0.21)	0.09 (0.04–0.18)	0.185	0.022

The values are geometric mean (95% CI).

*One-factor ANOVA repeated analysis.

† P -value for the treatment/time interaction (differences between groups).

Discussion

Peach allergy in the Mediterranean area is the flagship of LTP-related food allergies. It is a highly prevalent,

persistent and potentially severe food allergy (2, 3, 7, 9, 11–13), and accordingly a prime target for development of a curative treatment. In this exploratory clinical trial of immunotherapy for peach allergy, we aimed at

Table 4. Summary of adverse reactions

	Doses administered				Adverse events	Adverse reactions deemed related to SLIT					
	Build-up phase	1st week maintenance	Home maintenance	Total		Total	Total	Local reactions		Systemic reactions	
								N	Patients (%)	N	Patients (%)
Active (<i>n</i> = 37)	495	169	2714	3378	1420	1344	1328	33/37 (89.2)	16	5/37 (13.5)	
Placebo (<i>n</i> = 18)	246	78	1242	1566	60	12	9	5/18 (27.8)	3	3/18 (16.7)	

N is the total number of reactions.

establishing whether the dose, schedule and length of the treatment could lead to clinical efficacy and immunological changes without safety concerns.

We have found that the actively treated patients tolerated at least three times (three to nine times) more peach in the DBPCFC after 6 months of SLIT, had a 50% reduction in the rate of SRs, presented a significant decrease in SPT reactivity, and a significant increase in the IgG₄ responses to Pru p 3, whereas no differences were observed within the placebo group from T0 to T6. The increase in the IgE value to Pru p 3 observed in both study arms at T1 is probably related to the DBPCFC with peach performed at T0. An increase was still observed in both arms at T6 but only in the active group the titre was significantly higher over baseline. In summary, significant changes in the outcome of the DBPCFC and in the immunological parameters were observed in the active group. However, inter-group differences were only found in the SPT and IgG₄ responses. For the DBPCFC, the comparison of the survival curves of SRs at T6 showed almost significant differences ($P = 0.06$) between groups, but the discrete-time survival analysis did not. The originally planned statistical analysis of DBPCFC in this trial was the survival analysis (Log Rank test), but on the basis of the publication of Chinchilli et al. (25) of the discrete-time survival analysis, best suited to these data, prompted us to perform it additionally.

Failure to demonstrate significant differences in the DBPCFC between groups can be related to several factors including the methodology of the DBPCFC and its statistical analysis, the dose of allergen given and/or the duration of the treatment.

The DBPCFC is a rigorous tool to evaluate the clinical reactivity of patients reporting adverse reactions to foods (19) that has been applied in this trial to evaluate the efficacy of SLIT. The current experience in the use of DBPCFC to evaluate efficacy of therapies for food allergy is very limited (27, 28), and the related statistical issues have recently been highlighted and reviewed (25). Because of the clinical presentation of peach allergy in our population, and for safety concerns, the responses in the DBPBFC were classified into local and systemic. Having two possible outcomes may have hindered the evaluation

of the changes in DBPCFC, and future confirmatory trials should be focused at only one outcome.

After 6 months of SLIT, the patients in the active group received an average cumulative dose of Pru p 3 of 948 µg. In a previous 12 week SLIT trial with hazelnut (28), the active treatment was proved effective by DBPCFC although no changes were observed in the SPT or serum IgE responses. We have estimated that the dose of hazelnut LTP (Cor a 8) was probably twice the Pru p 3 dose given in our trial. A higher home maintenance dose in our trial would have been probably more effective, and should be increased in any future trials in the light of our tolerance results. With the scarce experience in SLIT in food allergy (28–30) it is not possible to establish at present the optimal dose and duration of treatment. This is not even fully established in respiratory allergy in which there is much larger experience with SLIT (31). Sublingual immunotherapy with grass tablets (GRAZAX) has demonstrated efficacy in grass pollen allergic rhinoconjunctivitis after 6 months of daily administration of 15 µg of Phl p 5 (cumulative dose over 2700 µg) (32), with a further progressive effect in immunological parameters and clinical outcome after 2 years of continuous treatment (33). In contrast, effective SLIT in mite allergy has been reported with much lower annual doses of major allergens: 116.5 µg of group 1 and 58.2 µg of group 2 allergens (34).

Sublingual immunotherapy with peach has proved to be safe as no serious adverse events were observed. Furthermore, mild SRs were observed with a similar frequency in both study arms (13.5% in the active group vs 16.7% in the placebo group), and they subsided either spontaneously or with oral antihistamines, and when gastric complaints were associated antacids and/or omeprazol were sometimes given. However, almost 90% of the patients in the active group presented LR in the oral cavity. Most of them consisted of mild oral itching that appeared predominantly during the build-up and first maintenance week. Subsequently, 40.5% of the patients did not refer to any complaint. Local oral reactions were well accepted by the patients probably because they were transient and of mild intensity. However, their frequency in this trial was higher than in the previous trial of hazelnut SLIT or in SLIT with

inhalants or latex (28–35), and might be related to the fact that the oropharyngeal mucosa is the target organ in most of the peach-allergic patients.

In summary, the results of this first exploratory clinical trial of SLIT for peach allergy show that it can be a promising therapeutic option that could modify the clinical reactivity of the patients to the intake of peach and the underlying immunological response with an overall good tolerance. Further studies are needed to

refine it, and to define the optimal allergen dose and duration of the therapy.

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