ORIGINAL ARTICLE

Sublingual Immunotherapy in Peach Allergy: Monitoring Molecular Sensitizations and Reactivity to Apple Fruit and *Platanus* Pollen

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Abstract

Background: Peach allergy is prevalent, persistent, and potentially severe and as such is a target for immunotherapy. Our aims were to evaluate the profile of sensitization to *Rosaceae* allergens and the effects of sublingual peach immunotherapy on immunoglobulin (Ig) E levels to these allergens, to monitor for neosensitizations, and to check if this treatment modified other *Rosaceae* fruit and pollen-related sensitizations.
Methods: A double-blind placebo-controlled trial was conducted on 56 peach-allergic patients who received, sublingually, a standardized peach extract quantified in mass units of Pru p 3, or placebo for 6 months. IgE to recombinant (r) Mal d 1, rMal d 4, rPru p 3, and natural (n) Art v 3 and skin prick test (SPT) reactivity to *Platanus* pollen and apple extracts evaluated before treatment (T0), after 1 month (T1) and after and 6 months (T6) were recorded.
Results: In total, 18.5% of patients recognized rMal d 1, 83.3%, rPru p 3, 24.1%, rMal d 4, and 25.9% nArt v 3. IgE to Pru p 3 rose from T0 to T1 in both the active group (P=.003) and the placebo group (P=.022), and remained elevated at T6 in the active group (P=.001). IgE to other purified allergens did not change significantly and no relevant neosensitizations were detected. SPT reactions to peach decreased from T0 to T6 in the active group (P<0.05). Reactivity to peach (T1 and T6) and apple (T6) was lower in the active group than in the control group.
Conclusions: The main allergen was Pru p 3. Changes in rPru p 3 IgE levels and in peach and apple extract SPT were induced by sublingual immunotherapy.

Key words: Food allergy. Immunotherapy. Sublingual immunotherapy. Food allergens. Peach. Food immunotherapy. Molecular allergens.

Resumen

Antecedentes: La alergia a melocotón es prevalente, persistente, potencialmente severa y por lo tanto una posible diana para inmunoterapia. Los objetivos del estudio fueron evaluar el perfil de sensibilización a alérgenos de Rosaceae, los efectos de la inmunoterapia sublingual con melocotón sobre los niveles de IgE a dichos alérgenos, detectar si se producen neosensibilizaciones y examinar si este tratamiento modifica las sensibilizaciones frente a otras frutas *Rosaceae* y pólenes relacionados.
Métodos: Se realizó un estudio doble ciego controlado con placebo en el que 56 pacientes alérgicos a melocotón fueron randomizados a recibir sublingualmente un extrato de melocotón cuantificado en unidades de masa de Pru p 3 o placebo, durante seis meses. Se registraron

los niveles de IgE a rMal d 1, rMAl d 4, rPru p 3 y nArt v 3 y la reactividad a manzana y polen de Platanus en prick, en evaluaciones realizadas antes (T0), al mes (T1) y a los seis meses (T6) de tratamiento.
Resultados: Un 18,5% de los pacientes reconocieron rMal d 1, un 83,3% rPru p 3, un 24,1% rMal d 4 y un 25,9% nArt v 3. Los niveles de IgE a rPru p 3 aumentaron de T0 a T1 en el grupo activo (p=0,003) y placebo (p=0,022), manteniéndose elevados en T6 en el grupo activo (p=0,001). La concentración de IgE a otros alérgenos purificados no cambió significativamente. No se desarrollaron neosensibilizaciones relevantes. La reactividad cutánea a melocotón (T1 y T6) y a manzana (T6) fue menor en el grupo activo que en el control.
Conclusiones: El principal alérgeno fue Pru p 3. La inmunoterapia sublingual indujo cambios en los niveles de IgE a rPru p 3 y en la reactividad cutánea a melocotón y manzana

Palabras clave: Alergia alimentaria. Inmunoterapia sublingual. Alérgenos alimentarios. Melocotón. Inmunoterapia con alimentos. Alérgenos moleculares.

Introduction

Known food allergens from *Rosaceae* fruits belong to 4 groups of proteins: Bet v 1-homologous allergens [1,2], thaumatin-like proteins [3], nonspecific lipid transfer proteins (nsLTPs) [4], and profilins [1,5-7]. Several of these allergens are responsible for cross-reactivity between both closely related and distant species [8-10].

Component-resolved diagnosis applied to *Rosaceae* fruit allergy has revealed several sensitization patterns in different geographical areas conditioned by variations in environmental exposures. In Central and Northern Europe, the major allergens responsible for *Rosaceae* allergy are Bet v 1 homologues [9] and profilins [11]. Serum immunoglobulin (Ig) E from fruit-allergic patients recognizes both groups of allergens as a consequence of their cross-reactivity with allergens from birch pollen, a primary sensitizer [12]. In Southern Europe, in contrast, *Rosaceae* fruit allergy presents with or without associated pollen allergy [13], with Pru p 3 being the major allergen and primary sensitizer [7,9,13]. Profilin is considered a minor allergen within this group [7] and sensitization is restricted to patients with pollinosis, usually to grass pollen. These assorted molecular sensitization profiles determine different clinical risks, which vary according to the biochemical and physicochemical properties of the allergens involved; specifically, sensitization to stable allergens such as nsLTPs is associated with a higher risk of systemic reactions [7,9].

Allergenic nsLTPs have been identified in numerous plant foods, latex, tree leaves, and pollens [14-16]; of these *Artemisia* (Art v 3) [10,17,18] and *Platanus* (Pla a 3) [19, 20] nsLTPs have demonstrated cross-reactivity with nsLTPs from foods.

The use of purified allergens in follow-up immunotherapy studies allows the measurement of immune response to individual allergen components during treatment. It also permits the detection of neosensitizations, which have been mainly described during subcutaneous immunotherapy (SCIT) with complete extracts [21]. Very few studies, however, have explored neosensitizations in sublingual immunotherapy (SLIT) [22].

We conducted a double-blind placebo-controlled trial in which peach-allergic patients were randomized to receive, sublingually, a standardized peach extract quantified in mass units of Pru p 3, or placebo for 6 months [23]. The aim of the present study was to evaluate the profile of sensitization to allergens from *Rosaceae* fruits in the study population, to monitor outcomes, and to detect possible neosensitizations during the course of SLIT. In addition, in order to check the effect of peach SLIT on response to other *Rosaceae* fruits and pollen-related sensitizations, we measured specific immunoglobulin (Ig) E to Art v 3 (LTPs from *Artemisia*) and evaluated changes in skin reactivity to *Platanus* pollen and apple during this treatment.

Material and Methods

Patients and Study Protocol

Fifty-six peach-allergic patients were enrolled from the allergy departments of the Fundación Hospital Alcorcón in Madrid, Spain and Hospital Virgen del Camino in Pamplona, Spain and randomized at a ratio of 2 to 1 to receive sublingual peach extract or placebo for 6 months according to a double-blind study design. In order to ensure a similar distribution of patients reporting systemic reactions upon peach ingestion in the 2 arms of the study, stratified blocked randomization was carried out.

Patients provided written informed consent before recruitment. The clinical trial was approved by independent ethics committees from the 2 participating centres and by the Spanish Drug Agency. The trial was also conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

The inclusion criteria were age of 18 to 65 years; a positive history of allergy to peach ingestion; specific IgE to peach proven by a positive skin prick test (SPT) (≥3 mm wheal diameter) to either a commercial peach extract (ALK-Abelló, S.A, Madrid, Spain) or fresh peach (prick to prick technique) and/or by a peach CAP ≥0.70 kU/L; and a positive double-blind, placebo-controlled food challenge (DBPCFC) with peach according to a previously described procedure [23]. Exclusion criteria were a positive DBPCFC with placebo; a history of food allergic reactions with hypotension; a history of allergy to coconut (used for masking peach in DBPCFC); pollen immunotherapy in the preceding 2 years; any clinical condition that contraindicates immunotherapy according to the European Academy of Allergology and Clinical Immunology (EAACI) Position Paper on Immunotherapy [24]; any significant clinical condition that according to the investigator's judgement might have hampered patient safety

or study outcomes; and the inability of the patient to comply with the scheduled visits.

Data including a history of a systemic or local reaction to peach and of pollinosis were recorded. The outcomes evaluated were SPT response to *Platanus* pollen and apple extracts, and titers of specific IgE to recombinant (r) Mal d 1, rMal d 4, rPru p 3 and natural (n) Art v 3 evaluated at baseline (T0), after 1 month of treatment (T1), and at the end of the trial, after 6 months of treatment (T6).

Extracts and Purified Allergens

Peach extract was obtained from fresh peelings and quantified in micrograms of the major allergen Pru p 3 as described in Duffort et al [25]. As has been shown, in addition to Pru p 3, peach-peel extracts contain a certain amount of Pru p 1 and profilin [16]. This material was used to prepare the immunotherapy treatment as well as the DBPCFC and SPTs.

The SLIT extract was prepared as a glycerinated, phenolated saline solution of peach extract. The placebo preparation for immunotherapy was a glycerosaline phenolated solution identical to the active product except in allergen content. The immunotherapy regimen has been previously described [23].

The material for the DBPCFC was sterile lyophilized peach extract, bottled in pre-weighted amounts for the challenge doses.

Peach, *Platanus acerifolia* pollen, and apple extracts used for SPT were supplied by ALK-Abelló S.A. and tested in different concentrations: 50, 10, 2 and 0.4 µg/mL Pru p 3 for peach; 150, 30, and 6 histamine equivalent prick units for *Platanus*, and 6.25%,1.25%, and 0.25% for apple extract.

Recombinant Mal d 1, Mal d 4, and Pru p 3 were produced as previously described [26,27]. Natural Art v 3 was isolated according to the method described by Díaz-Perales et al [10].

Skin Prick Tests

SPTs were performed at T0, T1 and T6, and 10 mg/mL histamine dihydrochloride and saline were used as positive and negative controls, respectively. In patients with a positive history of peach allergy with a negative peach-extract SPT, prick to prick tests using fresh fruits were used. SPTs were performed in duplicate on the volar surface of the forearm by the same investigator throughout the study, following the recommendations of the EAACI [28]. Skin responses were recorded at 15 minutes, and the wheal areas were measured by planimetry. Changes in response were determined by Parallel Line Assay, as described by Martin et al [29].

Specific IgE to rMal d 1, rMal d 4, rPru p3, and nArt v 3

Blood samples were collected at T0, T1, and T6. Serum samples were stored at –20ºC and processed together at the Research Department of ALK-Abelló S.A. in Madrid, Spain at the end of the study. Specific IgE concentrations to rMal d 1, rMal d 4, rPru p 3, and nArt v 3 were determined by the ADVIA Centaur (Bayer HealthCare Diagnostics Division) immunoassay system developed by ALK-Abelló according to

Table 1. Immunoglobulin (Ig) E Recognition Profile Before Treatment

	All (n=54)		Systemic Reaction (n=21)		No Systemic Reaction (n=33)		Pollinosis (n=32)		No Pollinosis (n=22)		Active Group (n=37)		Placebo Group (n=17)	
	No. (%)[a]	GM (CI)[b]	No. (%)[a]	GM (CI)[b]	No. (%)[a]	GM (CI)[b]	No. (%)[a]	GM (CI)[b]	No. (%)[a]	GM (CI)[b]	No. (%)[a]	GM(CI)[b]	No. (%)[a]	GM (CI)[b]
rMal d 1	10 (18.5)	1.5 (0.6-3.8)	2 (9.5)	0.6 (0.5-0.6)	8 (24.2)	1.9 (0.6-5.9)	9 (28.2)	1.6 (0.6-4.5)	1 (4.5)	0.9-	6 (16.2)	1.7 (0.4-7.1)	4 (23.5)	1.2 (0.1-10.8)
rPru p 3	45 (83.3)	5.6 (4.3-8.3)	20 (95.2)	6.7 (3.9-11.4)	25 (75.8)	5.5 (3.6-8.4)	26 (81.2)	6.6 (4.0-10.8)	19 (86.4)	5.2 (3.5-7.8)	30 (81.1)	6.2 (4.2-9.4)	15 (88.2)	5.5 (3.0-10.0)
rMal d 4	13 (24.1)	3.1 (1.5-6.4)	3 (14.3)	1.2 (0.3-4.4)	10 (30.3)	4.0 (1.6-9.9)	12 (37.5)	3.4 (1.6-7.3)	1 (4.6)	0.9-	10 (27)	2.6 (1.1-5.8)	3 (17.6)	5.5 (0.1-271)
nArt v 3	14 (25.9)	2.2 (1.1-4.6)	5 (23.8)	3.5 (0.4-32)	9 (15.2)	1.7 (0.8-3.5)	9 (28.1)	3.9 (1.5-10)	5 (22.7)	0.8 (0.4-1.6)	13 (35.1)	2.0 (0.9-4.5)	1 (5.9)	

[a]Number (%) of patients with positive determination.
[b]Geometric mean (95% confidence interval) of specific IgE concentration (kU/L).

a previously published procedure [30]. Levels of ≥0.35 kU/L were considered positive.

Statistical Analysis

New sensitizations that appeared during treatment and differences in allergen recognition patterns between patients classified according to their clinical characteristics and treatment group were analyzed by the χ^2 or Fischer exact test. IgE concentrations to recombinant allergens were logarithmically transformed before analysis of changes during treatment by analysis of variance for repeated measures. Changes in immediate skin reactivity were analyzed by parallel-line assay and expressed as a Cutaneous Tolerance Index (CTI), which is the ratio of allergen extract concentrations eliciting the same skin response. For all the analyses, *P* values of lower than .05 were considered significant.

Results

Baseline Sensitization Profiles

IgE against rMal d 1, rPru p 3, rMal d 4, and nArt v 3 were determined in serum samples from 54 of the 56 randomized patients at the beginning of the study. As is shown in Table 1, rMal d 1 was recognized by 18.5% of patients, rPru p 3 by 83.3%, rMal d 4 by 24.1%, and nArt v 3 by 25.9%. The percentage of patients with positive IgE to rPru p 3 was slightly higher in the group of patients with a history of systemic reactions after peach intake than in the group of patients with just local reactions (95.2% vs 75.8%). Although this difference showed a tendency, it did not reach statistical significance (*P*=.075 by Fisher exact test). The association between rMal d 1 and rMal d 4 and the history of local or systemic reactions to peach intake did not reach statistical significance (*P*=.2). Patients with hay fever showed a slightly but not significantly higher prevalence of sensitization to rMal d 1 (28.2% compared to 4.5% in non-pollinic patients, *P*=.072); the corresponding percentages for sensitization to rMal d 4 were 37.5% and 4.6%, respectively (*P*=.019). Sensitization to rPru p 3 was similar in both groups. The only statistically significant difference between the active and placebo treatment groups for specific IgE levels to these allergens was for nArt v 3 (*P*=.04).

When IgE levels to rPru p 3 and nArt v 3 were compared in patients sensitized to nsLTPs (n=45), significantly higher levels to Pru p 3 were detected (geometric means 3.18 vs 0.26 kU/L, *P*<.001). A correlation between both nsLTPs was found (Spearman r=0.449, *P*<.001).

Monitoring Specific IgE to rMal d 1, rPru p 3, rMal d 4, and nArt v 3 During Immunotherapy

The results of specific IgE determination against rMal d 1, rPru p 3, rMal d 4, and nArt v 3 during immunotherapy are shown in Table 2. As previously reported [23], increased IgE levels to Pru p 3 at T1 compared to baseline were observed in both the active and placebo group (*P*=.003 and *P*=.022 respectively); these remained significantly elevated at T6 in the active group only (*P*=.001). No significant changes in specific

Table 2. Allergen-Specific Immunoglobulin E Levels (kU/L) Before (T0), 1 Month After (T1) and 6 Months After (T6) Sublingual Immunotherapy With a Peach Allergen Extract

		rMal d 1			rPru p 3			rMal d 4			n Art v 3		
		T0	T1	T6	T0	T1	T6	T0	T1	T6	T0	T1	T6
Active group (n=33)[a]	GM	0.17	0.19	0.18	2.70	4.35	4.23	0.25	0.24	0.25	0.32	0.37	0.35
	95% CI	0.11-0.27	0.12-0.29	0.12-0.28	1.41-5.15	2.13-8.92	2.12-8.44	0.14-0.44	0.14-0.42	0.14-0.44	0.20-0.53	0.22-0.63	0.21-0.59
Placebo group (n=16)[a]	GM	0.19	0.18	0.18	3.26	4.59	4.04	0.21	0.21	0.22	0.15	0.18	0.16
	95% CI	0.10-0.37	0.10-0.35	0.10-0.33	1.32-8.08	1.69-12.46	1.49-10.92	0.09-0.52	0.09-0.52	0.08-0.57	0.09-0.25	0.10-0.30	0.09-0.30

Abbreviations: GM, geometric mean; CI, confidence interval.
[a]Complete cases.

Table 3. Skin Reactivity Intragroup (Active and Placebo Treatment) Changes: Cutaneous Tolerance Index (95% Confidence Intervals)

	Active Group		Placebo Group	
	T0-T1	T0-T6	T0-T1	T0-T6
Peach	0.95 (0.61-1.48)	1.83[a] (1.16-2.87)	0.8 (0.43-1.51)	0.87 (0.48-1.55)
Apple	0.98 (0.52-1.85)	0.90 (0.51-1.58)	1.39 (0.71-2.74)	0.99 (0.41-2.39)
Platanus pollen	0.93 (0.48-1.78)	1.00 (0.62-1.63)	1.24 (0.66-2.32)	1.55 (0.29-8.33)

Abbreviations: T0, before treatment; T1, 1 month after treatment; T6, 6 months after treatment.
[a]$P<.05$

Table 4. Skin Reactivity Intergroup (Active Versus Placebo Treatment) Comparison: Cutaneous Tolerance Index (95% Confidence Interval)

	T0	T1	T6
Peach	0.45 (0.15-1.23)	0.39[a] (0.15-0.92)	0.19a (0.07-0.47)
Apple	0.55 (0.15-1.65)	0.45 (0.14-1.28)	0.37a (0.11-0.95)
Platanus pollen	1.64 (0.32-12.17)	3.72 (0.66-78.31)	1.49 (0.42-6.23)

Abbreviations: T0, before treatment; T1, 1 month after treatment; T6, 6 months after treatment.
[a]$P<.05$

IgE to rMal d 1, rMal d 4, or nArt v 3 were found in either the active group or the placebo group.

During the follow-up period, 3 patients in the active group developed new sensitizations to rMal d 1, rPru p 3, and rMal d 4 (1 patient to each allergen). Concentrations of specific IgE were 0.47, 0.39, and 0.49 kU/L respectively. None of these patients had a worse DBPCFC response at T6 compared to T0. No neosensitizations were found in the patients in the placebo group.

Changes in Skin Reactivity

Changes in skin reactivity to peach, apple and *Platanus* pollen extracts are expressed as CTI and shown in Table 3. As described elsewhere [23], skin reactivity to peach extract was lower at T6 than at T0 in the active group (CTI, 0.83; 95% confidence interval [CI], 1.16-2.87; $P<.05$). No significant differences were found in changes in skin reactivity to either apple or *Platanus* pollen extracts in the active group. No significant changes in skin reactivity were detected in the placebo group either.

Reactivity to peach extract at T1 and T6 and to apple at T6 (Table 4) was significantly lower in the active than in the placebo group. No differences were observed for *Platanus* pollen skin response.

Discussion

We performed a double-blind placebo-controlled study in order to evaluate both clinical patterns (efficacy and tolerance) and immunologic patterns. This design allowed us to distinguish between immunologic changes caused by the spontaneous development of molecular sensitizations and/or DBPCFC exposure from those induced by immunotherapy.

Specific IgE determinations were performed with Mal d 1 (Bet v 1-homologue) and Mal d 4 (profilin), characterized in apple but with a high homology with Pru p 1 and Pru p 4 from peach, respectively [6,31]. We measured specific IgE levels to Mal d 1 (Bet v 1-homologue) and Mal d 4 (profilin) as well as to Pru p 3 and Art v 3 for 2 reasons. Firstly, these tests allowed us to evaluate the baseline sensitization profile in our patients and to explore the existence of differences between different clinical groups of patients (systemic vs nonsystemic reactors, with or without pollinosis). Secondly, they allowed us to check whether or not specific immunotherapy with an extract of peach peel was capable of modifying sensitization to these allergens.

The extract used for both DBPCFCs and SLIT was standardized for Pru p 3 content. Pru p 1 and Pru p 4 content was not determined but extracts obtained using the same method contain a certain amount of Pru p 1 and profilin [16]. Since the same extract was used for SLIT and DBPCFC, patients with a positive DBPCFC without sensitization to Pru p 3 were not excluded.

As previously described in other studies conducted in Southern Europe [7,9,13,32], the main allergen in our patients was Pru p 3, particularly in patients with a history of systemic reaction without associated pollinosis. Nevertheless, and in contrast with reports from other studies [32], Pru p 3 was also the most important sensitizer in our nonsystemic reactors and patients with associated pollinosis, although the frequency of sensitization to Bet v 1-homologue and profilin was also increased in these cases. Patients sensitized to Bet v 1-homologue were mostly in Pamplona, in the north of Spain, where several plants from the Fagales order are common. Similar results have been reported by Gamboa et al [32] for a nearby area.

This is the first time that *Rosaceae* molecular allergen sensitization has been monitored after DBPCFC and during specific immunotherapy. We previously showed increased IgE to rPru p 3 in both the active and placebo groups at T1, probably as a consequence of exposure to a high dose of complete peach extract in the DBPCFC [23]. This effect was not seen for IgE to rMal d 1 or rMal d 4, possibly because of the amount of allergen contained in the extract used [16] and/or their lability. Only the SLIT-treated group still had increased IgE levels to Pru p 3 at T6. No changes in IgE to Mal d 1 or Mal d 4 were found in the 6 months of study.

Just 3 patients in the active group developed new sensitizations (each to a single allergen). All of the sensitizations were of scarce magnitude and clinically irrelevant, as confirmed by DBPCFC after 6 months of SLIT. These findings are in accordance with those of other authors who have monitored allergen sensitization during SCIT [21] and SLIT [22]. With some exceptions [33], new IgE specificities that develop during immunotherapy are generally not clinically relevant.

In patients treated successfully with subcutaneous and sublingual immunotherapy to pollen, a beneficial response in terms of food-related allergy has been claimed by some authors [34-36] but not by others [37-39]. Until now, the effect of food immunotherapy on other food or pollen-related sensitization had not been evaluated. We found some effect on skin reactivity to apple after 6 months of treatment in the active compared to the placebo group. No effect of sublingual peach immunotherapy on cutaneous reactivity to *Platanus* pollen was detected.

In conclusion, the main allergen in our patients was Pru p 3, even in nonsystemic reactors and patients with associated pollinosis. Changes in IgE levels to rPru p 3 (but not to rMal d 1 or rMal d 4) and in peach and apple extract SPT results were induced by SLIT.

Potential Conflicts of interest

Domingo Barber, Santiago Martín and Pilar Rico work for ALK-Abelló, S.A, which provided funding for this study.

Funding

The clinical trial described was sponsored by ALK-Abelló S.A. in Madrid, Spain

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Manuscript received March 13, 2010; acccepted for publication June 22, 2010.

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