

Comparative Study of Tolerance between Unmodified and High Doses of Chemically Modified Allergen Vaccines of *Dermatophagoides pteronyssinus*

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Key Words

Immunotherapy · Depigmented-polymerized allergen extracts · *Dermatophagoides pteronyssinus* · Tolerance

Abstract

Background: The physiochemical modification of allergens to reduce allergenicity, while retaining immunogenicity, provides a chance for the administration of higher doses of immunotherapy, with a decreased risk of systemic reactions. **Objective:** To evaluate the safety of doses of depigmented glutaraldehyde-polymerized vaccine of *Dermatophagoides pteronyssinus* increasing those used in normal clinical conditions in comparison with regular doses of a non-modified vaccine. **Materials and Methods:** The study was double-blind, parallel and included two patient groups. Twenty-three patients were treated weekly during 9 visits for the build-up phase, followed by 2-weekly maintenance doses (a total of 11 injections per patient). Eleven patients (mean age 22 years) received immunotherapy with the standardized modified vaccine. The maximum dose used was the result of depigmenting and polymerizing 100 times the maximum dose used with the native extract. The maximum concentration used was 990 µg/ml of freeze-dried material. Twelve patients (mean age 24 years) received a stan-

dardized, unmodified allergenic extract. The maximum concentration used was 70 µg of freeze-dried material/ml, with a potency of 10 HEP_L/ml. The tolerance was evaluated recording all the side reactions related to immunotherapy and graded following the recommendations of the European Academy of Allergology and Clinical Immunology. **Results:** In both groups, all immediate local reactions were clinically not relevant. Patients treated with the native extract experienced 42 local immediate and 24 delayed reactions (1 of them had a diameter >10 cm), whereas patients treated with the modified extract experienced 30 local immediate and 21 delayed reactions (2 of them had a diameter >10 cm). Four systemic reactions in 2 patients, 1 immediate of grade 1 and 3 delayed of grade 2 were reported in the group treated with native extract and 1 delayed of grade 2 in the group treated with the modified preparation. **Conclusions:** The modified extract of *D. pteronyssinus*, used at concentrations which are 10 times higher than those regularly administered in clinical practice, demonstrated to be safe to treat *D. pteronyssinus*-sensitive patients. The majority of the local reactions (immediate and delayed) were clinically not relevant (diameter <5 cm). No systemic reactions of grade 3 or 4 were noted.

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Introduction

Allergen immunotherapy is the practice of administering gradually increasing quantities of an allergen extract to an allergic subject to ameliorate the symptoms associated with the subsequent exposure to the causative allergen [1] and is the only recognized treatment that can alter the natural course of allergic diseases [2]. Vaccines are used in medicine as well as in allergen immunotherapy as immune modifiers, because they modify or downregulate the immune response for allergic diseases, being part of a broad-based category of therapy presently used to treat immunologic and infectious diseases [1]. Treatment with allergen vaccines of house dust mites has been shown to produce clinical benefit in double-blind, placebo-controlled clinical trials [1, 3, 4] and in open randomized studies. In most studies using specific bronchial challenges as an objective measure of efficacy with mite vaccines, the threshold dose eliciting an immediate bronchial obstruction following immunotherapy was significantly increased [1]. Furthermore, mite immunotherapy has shown to reduce symptoms and/or the need for asthma medications [1]. To achieve this goal, a series of injections over a long period of time is required. This long-lasting treatment can be considerably shortened using higher doses [5, 6], although this approach is more dangerous since a relationship between the dose administered and the appearance of adverse effects has been established [7, 8]. However, it has been shown that efficacy is dose dependent, thus, the optimal dose has to be assessed, which is defined as the dose of allergen vaccine inducing a clinically relevant effect in most patients without causing unacceptable side effects [9, 10].

In an attempt to overcome this disadvantage, glutaraldehyde-modified allergenic extracts were developed, proving to be safer than unmodified vaccines while retaining their clinical efficacy [11, 12]. The introduction of a depigmentation step before the polymerization process removes adsorbed pigments, enhances the solubility of the allergoid and inactivates the enzymatic activity [13]. This recently developed methodology has been applied to extracts used in several clinical and in vitro studies [14–16]. These vaccines maintain or increase the immunogenicity and decrease specific IgE binding, facilitating the administration of higher doses compared to those used with non-modified allergens and shortening the build-up phase.

The safety and efficacy of these modified allergen extracts have been proven for house dust mites [17], *Parietaria judaica* [14], *Olea europaea* [18] and *Phleum*

pratense [16], using chemically modified doses exceeding 10 times the regular doses of non-modified vaccines.

The objective of this study was to evaluate the effect of treatment with a depigmented glutaraldehyde-polymerized vaccine of *Dermatophagoides pteronyssinus* with a dose that is 10 times higher than the dose used in normal clinical practice and has demonstrated efficacy in the studies mentioned in the previous paragraph. These doses were obtained by 100-fold chemical treatment of the doses that are used regularly with non-modified vaccines. Following the recommendations of the Committee for Proprietary Medicinal Products for the clinical evaluation of new vaccines [19], we compared the modified doses with the normal clinical doses of the respective non-modified vaccine.

Patients and Methods

Study Design

The study was double-blind, parallel and included two patient groups. Patients in group A were treated with standardized unmodified allergen extract, and those in group B received standardized modified extract at doses that were 10 times higher than those used regularly in clinical practice, thus the safety profile of these clinically regular doses could also be evaluated. The study was approved by the Ethics Committee of the Hospital 'La Fe' (Valencia, Spain) and the Spanish Health Authorities (Agencia Española del Medicamento). All patients gave their written informed consent to participate in the study. The aim of the study was to evaluate adverse events due to immunotherapy during the buildup phase to reach the maximum-tolerated dose.

Patients

Patients were recruited from the outpatient clinic of the Allergy Department of the 'Hospital la Fe'. Twenty-three patients (8 men and 15 women; mean age 23 years) were included in the study. All of them met the following inclusion criteria: rhinoconjunctivitis and mild or moderate bronchial asthma (diagnosed according to the guidelines of the Global Initiative for Asthma [20]) lasting more than 2 years, positive skin prick test (wheal size diameter ≥ 3 mm in the absence of a reaction with glycerinated saline solution) to a standardized *D. pteronyssinus* extract (100 HEP/ml; Laboratorios LETI) and negative to other common aeroallergens, and detectable specific IgE to *D. pteronyssinus*. The exclusion criteria were those outlined in the WHO position paper on allergen immunotherapy [1].

Patients were blindly randomized to one of the two groups. Group A consisted of 12 patients (2 males and 10 females, mean age: 24 years, range 19–36 years) who received treatment with the unmodified extract, and group B comprised 11 patients (6 males and 5 females, mean age 22 years: range 18–29 years) who received treatment with the modified extract.

Skin Prick Tests

Skin prick tests were performed at baseline and at the end of the study using the same batch of native unmodified allergen ex-

tract. The skin prick tests were conducted in duplicate, on the volar surface of the forearm using four 10-fold dilutions of native extract of *D. pteronyssinus* (highest concentration 2 mg of freeze-dried extract/ml, with a potency of 100 HEP/ml, containing 81.40 µg of Der p 1/ml and 59.2 µg/ml of Der p 2, Laboratorios LETI). The same batch of native, unmodified allergen extract was used throughout the trial. The extract was supplied freeze-dried and vacuum-packed to be reconstituted just before use. Histamine HCl 10 mg/ml and glycerinated saline solution were used as positive and negative controls, respectively. To avoid possible circadian differences in skin reactivity, skin tests were done between 9 and 11 a.m. by the same experienced technician. None of the patients had pretreatment with drugs that could affect the results of the test. All the reactions were recorded 15 min after application [21]. Wheal areas were outlined with a fine-tip marker and transferred, using transparent tape, to the corresponding sheet of the case report form. The area of each wheal was measured by planimetry using a Wacom palette (Wacom Technology, Vancouver, Wash., USA) and the computer program MacDraft (Microspot, Boca Raton, Fla., USA). For each patient, the individual bioequivalent dose of allergen extract to achieve a wheal of the same size as a positive control (10 HEP) was calculated [22].

Allergen Extracts for Immunotherapy

One hundred grams of a full-grown whole *D. pteronyssinus* culture were extracted 1:20 (w/v) in PBS followed by diafiltration in 3-kDa Pellicon membranes (Millipore, Madrid, Spain). Afterwards, the extract was freeze-dried, obtaining the native extract. The next step of depigmentation was conducted following a patented procedure [23]. Briefly, the native extract was reconstituted and incubated for a short period of time at pH 2. Afterwards, the extract was extensively dialyzed overnight in 3.5-kDa cutoff membranes. The pH gradually increased until the starting pH was reached. This procedure further removed pigments and originated in the depigmented extract. This depigmented allergen preparation was treated with glutaraldehyde to produce a polymer (modified extract), using previously described methods [24, 25]. This step was followed by dialysis to remove components with a molecular weight of less than 100 kDa.

SDS-PAGE electrophoresis was performed with native, depigmented and polymerized allergen extracts in order to monitor each step of the production process.

Major allergens, Der p 1 and Der p 2, were measured by ELISA using monoclonal antibodies (Indoor Biotechnologies, Charlottesville, Va., USA) according to the instructions of the manufacturer. The results were expressed as the quantity of major allergen (in µg) per mg of freeze-dried extract. The protein content was measured using the method of Bradford [26]. The protein nitrogen units were determined using the micro-Kjeldhal method.

The inhibition of specific IgE binding was evaluated by a capture-specific IgE-binding competition ELISA [27], and the IgG binding activity was measured by means of an ELISA-specific IgG inhibition assay [28]. In both cases, and following the recommendations of the European Pharmacopoeia [29], the in-house reference preparation of the native allergen extract was used as standard, and the results were expressed as the quantity (micrograms) of freeze-dried material added to the well of the microplate to achieve the 50% inhibition.

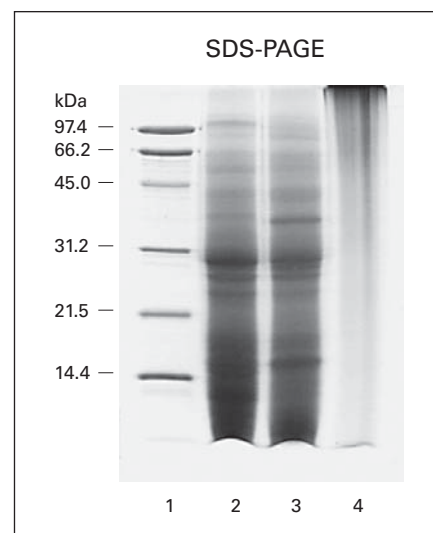


Fig. 1. SDS-PAGE electrophoresis pattern of the native (lane 2), depigmented (lane 3) and depigmented-polymerized (lane 4) preparations of *D. pteronyssinus*.

Allergen Vaccines and Immunotherapy Schedule

Each treatment set consisted of 3 numbered vials (1, 2 and 3) of 10-fold increasing doses of standardized native or modified extract adsorbed onto aluminum hydroxide. The allergen concentration in each treatment vial administered to group A was that regularly used in clinical practice, with the maximum concentration (vial No. 3) having a potency of 10 HEP_I/ml. In group B, patients received an allergen concentration which was 10 times higher than the one regularly used in clinical practice for this type of treatment. The maximum concentration (vial No. 3) was the result of depigmenting and polymerizing 1,000 HEP_I/ml (8,520 µg), having a residual potency of 3.51 HEP_I/ml due to the chemical modification, and vial No. 2 was the one of maximum concentration in normal clinical conditions. The residual potency was measured by ELISA competition [27], comparing the unmodified with the modified allergen extract. The native extract of *D. pteronyssinus* contained 20.35 µg of Der p 1 and 12.30 µg of Der p 2 per milligram of freeze-dried extract. Due to the polymerization process, Der p 1 and Der p 2 were not detectable in the modified extract. Figure 1 shows SDS-PAGE electrophoresis.

None of the patients received premedication with anti-allergic drugs before the administration of any dose. The subcutaneous administration was always under medical supervision. After each administration, the patient remained for at least 30 min in the center where the administration took place, in order to detect any local or systemic immediate adverse reactions. The patient was contacted by telephone or personal contact was established with respect to delayed adverse events.

The patients were instructed to immediately inform the physician about all adverse events, whether or not associated with immunotherapy.

Safety Monitoring

The study was explanatory and since all side effects were recorded, reactions that were considered as not relevant in normal

Table 1. Characteristics of the maximum concentration of native and modified vaccines

	Unmodified	Modified
Freeze-dried extract, µg/ml	85.2	990
Protein, µg/ml	70.0	900
PNU/ml	626	8,790
Der p 1, µg/ml	1.73	ND
Der p 2, µg/ml	1.05	ND
Potency, HEP _L /ml	10	3.51 ¹

ND = Not detectable due to the modification process. The initial native preparation contained originally 173 µg of Der p 1 and 105 µg of Der p 2.

¹ Residual potency after the modification process. The initial potency of the native extract was 1,000 HEP_L.

clinical conditions were recorded, too. Side effects due to immunotherapy were quantified according to the Position Paper of the European Academy of Allergology and Clinical Immunology [30]. The reactions were categorized as immediate when the onset was during the first 30 min following the administration, and delayed when the onset was after this time. Local reactions were quantified measuring the diameter of the reaction. Immediate reactions with a diameter of less than 5 cm and delayed reactions of less than 10 cm were considered as clinically 'not relevant'. The systemic reactions were graded as: *grade 0*: no symptoms; *grade 1*: unspecific symptoms – reactions probably not IgE mediated, i.e. discomfort, headache or arthralgia, for example; *grade 2*: mild systemic reactions – mild rhinitis or asthma responding adequately to antihistamines or β_2 -agonist therapy; *grade 3*: non-life-threatening systemic reactions – urticaria, angioedema, or severe asthma, responding well to treatment, and *grade 4*: anaphylactic shock – rapidly evoked reaction of itching, flushing, erythema or bronchial obstruction, for example, requiring intensive treatment.

Statistics

The study was designed as a pilot study. The sample size was chosen following the recommendations of André and Foulkes [31] for tolerance studies on the development of vaccines. Sample size was calculated on the basis that 80% of the total systemic reactions would occur in group A and 20% in group B. For an α of 0.05 and a power of 80%, the number of patients per group was 11. We decided to increase this number by 10% in order to cover possible dropouts. Thus, the final number of patients per group was 12. The software SPSS (SPSS, Chicago, Ill., USA) was used for statistical analysis. log-log regression analysis of the skin test dose-response bioassay was conducted [32]. The amount of allergen needed to produce a wheal of the same size as the histamine-positive control (10 HEP) was calculated for each patient [22]. The value of the median and the interquartile (IQ) range was used for descriptive statistics. The non-parametric Mann-Whitney test was used for intergroup comparisons, comparing the values of the individual 10 HEP at baseline and at the end. The Wilcoxon signed rank test was used for intragroup comparisons, comparing the values obtained at baseline with those obtained at the end. A contingency table was used to compare the number of adverse reactions between both groups.

Results

Characteristics of the Therapeutic Vaccines Used

The maximum concentration of both vaccines was 85.2 µg/ml for the unmodified extract and 990 µg/ml for the modified extract. These 990 µg/ml were the result of depigmenting and polymerizing 8,520 µg of unmodified extract. The 50% inhibition of the specific IgE binding for *D. pteronyssinus* was 295 ng for the native unmodified extract, being 18.52 µg (63 times more) for the resulting polymer. The amount of allergen extract needed to produce a 50% inhibition of specific human IgG binding was 13 ng for the native extract and 15 ng for the modified extract. The characteristics of the maximum concentrations of the vaccines containing the native and the modified extracts are shown in table 1.

Patients, Immunotherapy Schedule and Safety

Each patient received 11 injections. The total number of injections was 132 in group A, and 121 in group B. All patients reached the maximum dose previously established. Table 2 shows the up-dosing schedule used and the total accumulated dose.

Two patients in each group were free of local or systemic reactions. With the doses used in normal clinical conditions, 7 patients of group B were free of reactions. There were 66 local reactions in group A and 51 in group B ($p > 0.05$). Forty-two local immediate reactions occurred in group A patients, and 30 in group B ($p > 0.05$), with all being regarded as clinically irrelevant (diameter < 5 cm). There were 24 local delayed reactions in group A and 21 in group B ($p > 0.05$), showing 1 clinically relevant reaction in group A and 2 in group B (diameter = 10 cm; $p > 0.05$), the remainder of these reactions were clinically not relevant.

One immediate systemic reaction (grade 1) occurred in group A. Three delayed systemic reactions (all of grade 2) occurred in group A and 1 (grade 2) in group B. Local reactions are described and evaluated in table 3, and systemic reactions in table 4. Table 5 lists systemic (immediate and delayed) reactions and clinically relevant local reactions (diameter > 10 cm) per treatment vial.

Skin Prick Tests

The results are expressed as the amount of native, unmodified allergen extract necessary to induce a wheal equal in size to the positive control. At baseline, the group treated with native extract needed a median of 0.20 mg (IQ range 0.05–0.30) and the group treated with the modified extract 0.08 mg (IQ range 0.02–0.34). After the treat-

Table 2. Quantity of allergen extract and IgE potency (HEP_I) administered in each dose of the vaccine containing the native extract (group A), and data corresponding to the modified extract, describing the amount of modified allergen extract administered per dose injected, the original IgE potency (HEP_I) before the process of chemical modification and the residual potency after this process (group B)

Unmodified						Modified							
vial	units HEP _I /ml	dose	ml	µg/dose	HEP _I /dose	vial	potency, HEP _I /ml		dose	ml	µg/dose	HEP _I /dose	
							before modification	after modification				before modification	after modification
1	0.1	1	0.1	0.09	0.01	1	10	0.03	1	0.1	0.99	1	0.00
		2	0.3	0.26	0.03				2	0.3	2.97	3	0.01
		3	0.5	0.43	0.05				3	0.5	4.95	5	0.02
2	1	4	0.1	0.85	0.1	2	100	0.35	4	0.1	9.9	10	0.04
		5	0.3	2.56	0.3				5	0.3	29.7	30	0.11
		6	0.5	4.26	0.5				6	0.5	49.5	50	0.18
3	10	7	0.1	8.52	1	3	1,000	3.51	7	0.1	99	100	0.35
		8	0.3	25.56	3				8	0.3	297	300	1.05
		9	0.5	42.60	3				9	0.5	495	500	1.75
		10	0.5	42.60	5				10	0.5	495	500	1.75
		11	0.5	42.60	5				11	0.5	495	500	1.75
Total µg accumulated				170.31	1,979.01								

ment period, the results were 0.25 mg (IQ range 0.05–1.33) and 0.73 mg (IQ range 0.31–1.65), respectively. Figure 2 shows the box plot of these results. The statistical analysis (Mann-Whitney) showed that there were no statistically significant differences between the two groups ($p > 0.05$) at baseline and at the end. The intragroup comparison (Wilcoxon test) showed that the group treated with the modified extract needed a significantly ($p = 0.016$) higher quantity of allergen extract to achieve a wheal of the same size as the wheal of the positive control at the end of the study, whereas the group treated with the native extract remained without modification.

Discussion

In this paper, we present the tolerability of doses of a depigmented-glutaraldehyde polymerized vaccine of *D. pteronyssinus* that are 10 times higher than those used in normal clinical conditions. These doses are the result of chemically modifying 100 times the doses that are used regularly with non-modified vaccine. The regular clinical doses of the respective non-modified vaccine were used as comparator.

The study was conducted following the recommendations of the Committee for Proprietary Medicinal Prod-

Table 3. Local reactions classified according to the time of appearance: immediate (during the first 30 min) or delayed (30 min after treatment) and the size of the reaction

Wheal size	Immediate		Delayed	
	group A	group B	group A	group B
<5 cm	26	17	4	9
5–10 cm	0	0	7	7
>10 cm	0	0	1	2
NE	16	13	12	3
Total	42	30	24	21
NE = Not evaluated (<1 cm).				

ucts in the clinical evaluation of new vaccines [19] which indicates that, whenever feasible, besides an overall safety comparison, an additional very thorough comparison with the appropriate control vaccines should be made. Placebo was not used following the Note of Clarification on Paragraph 29 added by the World Medical Association General Assembly [33], which reaffirmed its position that extreme care must be taken in making use of a placebo-controlled trial and that in general this methodology should only be used in the absence of existing proven

Table 4. Systemic reactions: description of reactions, time of appearance (immediate or delayed), grade, dose that provoked the reaction, vial number, quantity injected and description

Patient ID	Group	Reaction	Grade	Dose No.	Vial No.	ml	Reaction/description
13198	B	delayed	2	9	3	0.5	bronchospasm; mild asthma 1 h after administration; the reaction disappeared spontaneously without treatment
72136	A	delayed	2	7	3	0.1	chest tightness for 2 days
72136	A	immediate	1	9	3	0.5	cough, chest tightness and rhinoconjunctivitis; the patient suggested air conditioning as a possible cause; clinical improvement after 30 min
92227	A	delayed	2	9	3	0.4	rhinitis
92227	A	delayed	2	10	3	0.4	rhinitis

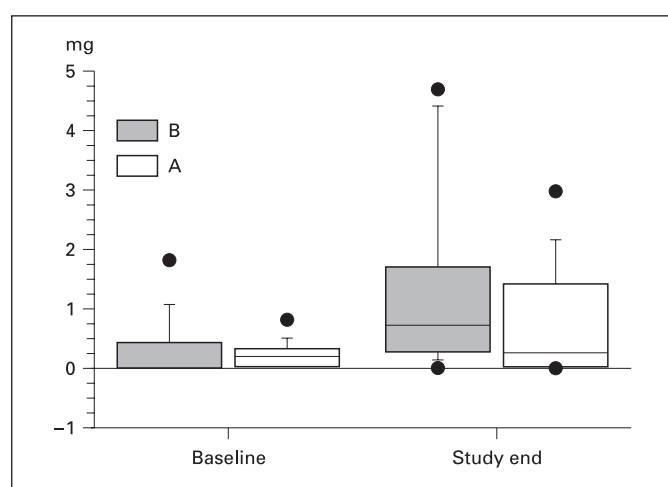


Fig. 2. Box plot view of the skin prick test results at baseline (before treatment) and at the end of the study.

therapy. The study was double-blind and designed to evaluate the safety profile of a modified allergen given as a therapeutic vaccine under experimental conditions only (explanatory clinical trial). Thus, all reactions were recorded, even those that are not documented in normal clinical practice.

In this study, 4 systemic reactions occurred in 2 patients following the vaccination with the unmodified preparation (3% of the injections). One, graded as 1, occurred with the maximum dose, the other 3 (graded as 2) occurred during the up-dosing process with the vial containing the maximum concentration. The patients treated with the vaccine containing the modified preparation experienced only 1 delayed systemic reaction (0.75% of the injections), graded as 2, with the injection of the planned maximum dose, which is 10 times higher than the maxi-

Table 5. Systemic (immediate and delayed) reactions and clinically relevant local reactions (diameter >10 cm) per vial

Reaction		Vial					
		unmodified			modified		
		1	2	3	1	2	3
Local							
	Immediate	0	0	0	0	0	0
	Delayed	0	0	1	0	0	2
Systemic							
	Immediate						
	Grade 2	0	0	0	0	0	0
	Grade 3	0	0	0	0	0	0
	Delayed						
	Grade 2	0	0	3	0	0	1
	Grade 3	0	0	0	0	0	0

Vials in bold show the maximum concentrations used regularly in the treatment of patients.

mum dose usually administered in normal clinical conditions.

Although the group of patients treated with the unmodified extract experienced more local reactions than group B (66 vs. 51), the differences were not significant ($p = 0.2560$). The majority of the local reactions (immediate and delayed) were clinically not relevant. Group B patients experienced 2 delayed local reactions with a diameter >10 cm following injection of the maximum planned dose.

Group B patients were also evaluated following treatment with the doses used in normal clinical practice. Neither systemic reactions nor relevant local reactions were recorded with these doses.

The maximum concentration of the unmodified therapeutic vaccine contained 1.73 $\mu\text{g}/\text{ml}$ of Der p 1 (in

85.2 µg of freeze-dried extract), being within the limits reported in the studies included in the Position Paper of the WHO [1, 5, 10, 34]. The changes induced by the chemical modification of the native extract did not allow measuring the contents of major allergen in the final product. Following the indications of the European Pharmacopoeia [29], the major allergens were measured in previous steps. In this case, the contents of major allergens in the native allergen extract, which was the precursor of the maximum concentration of the modified extract, were 173 of Der p 1 and 105 µg/ml of Der p 2.

Using native allergen extracts of mites in a dose-titration study, Olaguibel et al. [35] reported that the maximum-tolerated dose was between 0.8 and 16 µg of Der p 1, having a biological potency of 40 BU, with a frequency of systemic reactions per dose administered of 4.10%, with 82% of these reactions being of grade 1 or 2, and the remaining 18% of grade 3. The origin of the maximum dose of the modified extract was 4,260 µg of native extract, which contained 86.5 µg of Der p 1. This dose of the modified extract induced 1 systemic late reaction graded as 2 (0.83% per dose administered). It should be taken into consideration that the yield of the depigmented and polymerized allergen extract, in terms of freeze-dried material, is around 10% of the starting unmodified extract.

Other studies, including different non-modified allergen extracts, showed that immunotherapy was safe. Tabar et al. [36] performed a prospective safety study in 419 patients, mainly asthmatics sensitized to *D. pteronyssinus* (71%), treated in the immunotherapy unit according to a conventional schedule. They reported local reactions in 10.5% of the patients, and systemic reactions occurred in 0.37% of the doses administered, which corresponded to 4.8% of the patients, being mostly mild and delayed systemic reactions during the up-dosing period. In a follow-up study of 339 patients (75% mite-allergic patients, and 70.2% with rhinoconjunctivitis and asthma) treated in an immunotherapy unit, Sánchez et al. [37] reported 42 episodes of adverse reactions (0.8% of all the administered doses). Of these reactions, 36 (85.7%) occurred within 30 min after the injection was administered, consisting of 15 large local reactions (0.3% of the total amount of injection given) and 21 systemic reactions (0.4%) that occurred only in asthmatic patients. All the systemic reactions were mild and rapidly reversible with appropriate treatment. Only in 2 cases was an immediate systemic reaction associated with a large local reaction. Six reactions (14.3%) occurred after 30 min and consisted only of large local reactions. A total of 15 systemic episodes

(71.4% of all the systemic reactions) were registered at initial build-up doses. Using biologically standardized allergen vaccines which are commercially available, Moreno et al. [38] treated 433 patients (17,526 injections) according to a conventional schedule and reported 105 local reactions (0.6% per injection) in 48 patients and 53 systemic reactions (0.3% per injection) in 18 patients (3.7%; 30 of grade 2 and 21 of grade 3).

In our study, the doses of the modified extract used in the range of commercially available treatment sets produced no systemic reaction. Safety aspects of this modified preparation were investigated in a prospective multicenter observational cohort study [39] on 175 patients with rhinoconjunctivitis with or without asthma sensitized to grass pollen (55%), tree pollen (18%), a mixture of tree and grass pollen (14%), house dust mites (11%) and others (2%). A total of 2,261 injections were administered. Local reactions with a diameter >10 cm occurred in 0.1% of the injections. Thirteen patients experienced systemic reactions, all judged to be mild. Other studies have also demonstrated the efficacy of treatment with depigmented polymerized extracts of mites [17], *P. judaica* [14], *O. europaea* [18] and *P. pratense* [16] and the absence of moderate or severe systemic reactions.

The cutaneous reactivity was improved in group B, in which the patients, after reaching and repeating 2 times the maintenance dose, needed more quantity of allergen extract to achieve a wheal of the same size as the wheal induced by the positive control using 10 mg/ml histamine, whereas group A remained without modification. This fact indicates the quick establishment of an objective clinical benefit, probably due to the high doses of the modified therapeutic vaccine.

The study demonstrates that the depigmented and glutaraldehyde-polymerized allergen extract of *D. pteronyssinus*, used at concentrations which are 10 times higher than those regularly administered in clinical practice, is safe to treat *D.-pteronysinus*-sensitive patients, confirming the wide therapeutic margin for this chemically modified allergen vaccine and increased safety compared to regular vaccines based on native unmodified extracts.

References

- World Health Organization: Allergen immunotherapy: Therapeutic vaccines for allergic diseases. *Allergy* 1998;53(suppl):1–42.
- Moller C, Dreborg S, Ferdousi HA, Halken S, Host A, Jacobsen L, et al: Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 2002; 109:251–256.
- Abramson MJ, Puy RM, Weiner JM: Allergen immunotherapy for asthma. *Cochrane Database Syst Rev* 2000;CD001186.
- Abramson MJ, Puy RM, Weiner JM: Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. *Am J Respir Crit Care Med* 1995;151:969–974.
- Bousquet J, Calvayrac P, Guerin B, Hejjoui A, Dhivert H, Hewitt B, et al: Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. I. In vivo and in vitro parameters after a short course of treatment. *J Allergy Clin Immunol* 1985;76:734–744.
- García-Ortega P, Merelo A, Marrugat J, Richart C: Decrease of skin and bronchial sensitization following short-intensive scheduled immunotherapy in mite-allergic asthma. *Chest* 1993;103:183–187.
- Lockey RF, Benedict LM, Turkeltaub PC, Bukantz SC: Fatalities from immunotherapy (IT) and skin testing (ST). *J Allergy Clin Immunol* 1987;79:660–677.
- Committee on Safety of Medicines. Desensitizing vaccines. *BMJ* 1986;293:948–952.
- Creticos PS, Van Metre TE, Mardiney MR, Rosenberg GL, Norman PS, Adkinson NF Jr: Dose response of IgE and IgG antibodies during ragweed immunotherapy. *J Allergy Clin Immunol* 1984;73(1 pt 1):94–104.
- Haugaard L, Dahl R, Jacobsen L: A controlled dose-response study of immunotherapy with standardized, partially purified extract of house dust mite: Clinical efficacy and side effects. *J Allergy Clin Immunol* 1993;91:709–722.
- Grammer LC, Shaughnessy MA, Patterson R: Modified forms of allergen immunotherapy. *J Allergy Clin Immunol* 1985;76(2 pt 2):397–401.
- Bousquet J, Michel FB: Safety considerations in assessing the role of immunotherapy in allergic disorders. *Drug Saf* 1994;10:5–17.
- Berrens L, Gallego M, Boluda L, Fernández-Caldas E, González L: Complexed flavonoids on pollen protein allergens revealed by UV-spectroscopy and thin-layer chromatography. *Allergy* 1998;53(suppl):56.
- García-Selles J, Pascual A, Funes E, Pagan JA, Lopez JD, Negro JM, et al: Clinical efficacy and safety of a depigmented and glutaraldehyde polymerized therapeutic vaccine of *Parietaria judaica*. *Allergol Immunopathol (Madr)* 2003;31:63–69.
- Guerra F, Casanovas M, Daza JC, Almeda E, Ruiz A, Gómez MJ, et al: Safety and efficacy of immunotherapy with a standardized depigmented and glutaraldehyde-polymerized vaccine of *Olea europaea*. *J Allergy Clin Immunol* 2000;105:S312.
- Casanovas M, Gómez MJ, Cimarra M, Martínez-Cócerca C, Fernández-Caldas E: Immunotherapy with a standardized depigmented and glutaraldehyde-polymerized extract of *Phleum pratense*: Safety and efficacy. *Allergy* 2001;56(suppl 68):92.
- Ferrer A, García-Selles J: Significant improvement in symptoms, skin test, and specific bronchial reactivity after 6 months of treatment with a depigmented, polymerized extract of *Dermatophagoides pteronyssinus* and *D. farinae*. *J Investig Allergol Clin Immunol* 2003;13: 244–251.
- Guerra F, Daza JC, Almeda E: Immunotherapy with a depigmented, polymerized vaccine of *Olea europaea* pollen allergens significantly reduces specific bronchial and skin test reactivity in sensitized patients after one year of treatment. *J Investig Allergol Clin Immunol* 2003; 13:108–117.
- Committee for Proprietary Medicinal Products (CPMP): Note for guidance on clinical evaluation of new vaccines. <http://www.emea.eu.int/pdfs/human/ewp/046397EN.pdf> 1999.
- GINA: Global strategy for asthma management and prevention. NHLBI/WHO workshop report. NHLMI publication, 1995.
- EAACI. Position paper: Allergen standardization and skin tests. The European Academy of Allergy and Clinical Immunology. *Allergy* 1993;48(14 suppl):48–82.
- Nordic Council on Medicines: Registration of allergen preparations. Nordic guidelines. Prepared by the Nordic Council on Medicines in cooperation with the Drug Regulatory Authorities in Denmark, Finland, Iceland, Norway and Sweden. NLN Publication No. 23, 1989, January, pp 25–34.
- Berrens L: International Patent Application PCT. 1994, Publication No. WO 94/06821.
- Patterson R, Suszko IM, Zeiss CR, Pruzansky JJ: Development and evaluation of polymerized allergens for immunotherapy. *Int Arch Allergy Appl Immunol* 1981;66(suppl 1):293–296.
- Sachs DH, Winn HJ: The use of glutaraldehyde as a coupling agent for ribonuclease and bovine serum albumin. *Immunochemistry* 1970;7: 581–585.
- Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–254.
- Casanovas M, Marañón F, Bel I: Comparison of skin-prick test assay and reverse enzyme immunoassay competition (REINA-C) for biological activity of allergens. *Clin Exp Allergy* 1994;24:134–139.
- Boluda L, Gallego MT, Gonzalez Romano ML, Cambra O, Berrens L: Evaluation of the potency of allergenic extracts by inhibition of IgG-antibody binding. *Biologicals* 1994;22: 171–177.
- European Pharmacopeia. Allergen Products, ed 3. 1997.
- EAACI: Position paper: Immunotherapy. The European Academy of Allergy and Clinical Immunology. *Allergy* 1993;48(14 suppl):7–35.
- André FE, Foulkes MA: Criteria for progressing from phase I to phase III studies; in Plottkin S, Brown F, Hauraud F (eds): *Preclinical and Clinical Development of New Vaccines*. Basel, Karger, 1998.
- Finney DJ: *Statistical Methods in Biological Assay*. New York, Hafner, 1964.
- World Medical Association Declaration of Helsinki. 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002. Ethical Principles for Medical Research Involving Human Subjects. <http://www.wma.net/e/policy/pdf/17c.pdf>. 2002.
- Wahn U, Schweter C, Lind P, Lowenstein H: Prospective study on immunologic changes induced by two different *Dermatophagoides pteronyssinus* extracts prepared from whole mite culture and mite bodies. *J Allergy Clin Immunol* 1988;82(3 pt 1):360–370.
- Olaguibel JM, Tabar AI, García Figueroa BE, Cortés C: Immunotherapy with standardized extract of *Dermatophagoides pteronyssinus* in bronchial asthma: A dose-titration study. *Allergy* 1997;52:168–178.
- Tabar AI, García BE, Rodríguez A, Olaguibel JM, Muro MD, Quirce SA: Prospective safety-monitoring study of immunotherapy with biologically standardized extracts. *Allergy* 1993; 48:450–453.
- Sánchez I, García-Robaina JC, Bonnet C, de Blas C, Fernández-Caldas E, Sánchez M, et al: Immunotherapy units: A follow-up study. *J Investig Allergol Clin Immunol* 2001;11:167–171.
- Moreno C, Cuesta-Herranz J, Fernández-Távora L, Álvarez-Cuesta E: Immunotherapy safety: A prospective multi-centric monitoring study of biologically standardized therapeutic vaccines for allergic diseases. *Clin Exp Allergy* 2004;34:527–531.
- Sager A, Renner BG: Safety aspects and course of allergen-specific immunotherapy with a depigmented, polymerized allergen extract. *Allergo J* 2004;13:386–391.