ORIGINAL ARTICLE

Phase II/III Clinical Trial to Assess the Tolerability and Immunological Effect of a New Updosing Phase of *Dermatophagoides* Mix–Based Immunotherapy

Tabar AI[1], González Delgado P[2], Sánchez Hernández C[3], Basagaña Torrento M[4], Moreno Benítez F[5], Arina M[6]

[1]Hospital Virgen del Camino, Pamplona, Spain
[2]Hospital GU de Alicante, Alicante, Spain
[3]CPE Virgen de la Cinta, Huelva, Spain
[4]HU Germans Trias i Pujol, Badalona, Spain
[5]Clínica Dr. Lobatón, Cádiz, Spain
[6]Medical Department, ALK-Abelló, Madrid, Spain

Abstract

Background: Immunologically enhanced subcutaneous specific immunotherapy (SCIT) has been developed with a fast and simplified updosing phase containing equal parts of the house dust mites (HDM) *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (*Dermatophagoides* mix) adsorbed on aluminum hydroxide.
Objective: To evaluate the tolerability and immunological impact of the updosing phase of this new allergen extract formulation.
Material and Methods: We performed a multicenter, open-label, single-arm, phase II/III clinical trial. The inclusion criteria were a clinical history of rhinitis/conjunctivitis due to HDM (with/without asthma) and sensitization to HDM (positive specific IgE and skin prick test). Five updosing injections of *Dermatophagoides* mix (300, 600, 3000, 6000, and 15 000 SQ+) were administered at weekly intervals with 1 maintenance injection (15 000 SQ+) 2 weeks after the last updosing injection. Two days after each visit, patients were contacted by telephone to follow up on any adverse events. IgE-blocking factor, IgG4, and immediate skin reactivity were evaluated.
Results: The sample comprised 102 patients (mean [SD] age, 29.3 [7.7] years; male, 52.9%). There were 117 adverse drug reactions (ADR): 101 were local, regardless of reaction size, in 48 (47.1%) patients and 7 were systemic (all grade I) in 5 (4.9%) patients. All ADRs were mild, except for 1, which was moderate. Six weeks of treatment led to statistically significant increases in IgE-blocking factor and IgG4, as well as a significant reduction in immediate skin reactivity.
Conclusion: This new updosing phase of *Dermatophagoides* mix–based immunotherapy had a good tolerability profile and induced a significant immunological effect.

Key words: Specific immunotherapy. Allergens. House dust mites. Antigens. Immune response. Subcutaneous injection. Skin reactivity. Tolerability assessment.

Resumen

Antecedentes: Se ha desarrollado una mejorada vacuna de inmunoterapia específica (SCIT) adsorbida en hidróxido de aluminio y administración subcutánea con una fase de incremento de dosis más rápida y simplificada que contiene D. pteronyssinus y D. farinae (HDM; Dermatophagoides mezcla) a partes iguales.
Objectivo: Evaluar la tolerabilidad de la fase de incremento de dosis de esta nueva formulación de extracto alergénico en SCIT y su impacto inmunológico.
Material y Métodos: Ensayo clínico multicéntrico, abierto, de un brazo, fase II/III. Los sujetos que se podían incluir eran pacientes con una historia clínica de rinitis/conjuntivitis a ácaros de polvo doméstico (con/sin asma) y que presentaran sensibilización a HDM (IgE específica y prueba cutánea positiva). Se administraron cinco dosis semanales de Dermatophagoides mezcla en la fase de inicio (300, 600, 3000,

6000 y 15.000 SQ+) y una inyección de mantenimiento (15.000 SQ+) dos semanas tras la última inyección de la fase de incremento de dosis. Dos días tras cada visita se contactó con los pacientes por teléfono para seguir cualquier acontecimiento adverso (AE). Además, se evaluaron la IgG4, factor bloqueante de IgE y la respuesta cutánea inmediata.
Resultados: Se incluyeron 102 sujetos en el ensayo (52,9% varones) con una edad media de 29,3±7,7 años. Se notificaron 117 reacciones adversas (RA) relacionadas con el medicamento en investigación: 101 locales, con independencia del tamaño de la reacción, en 48 (47,1%) pacientes y 7 sistémicas, todas grado I, en 5 (4,9%) pacientes. Todas las RA fueron de intensidad leve, excepto una, de intensidad moderada. Tras seis semanas de tratamiento, se obtuvieron incrementos estadísticamente significativos en el factor bloqueante de IgE y en IgG4, así como en la reducción de la respuesta cutánea inmediata.
Conclusión: Esta nueva fase de incremento de dosis con inmunoterapia con Dermatophagoides mezcla presenta un buen perfil de tolerabilidad e induce una respuesta inmunológica significativa.

Palabras clave: Inmunoterapia específica. Alérgenos. Ácaros de polvo doméstico. Antígeno. Respuesta immune. Inyección subcutánea. Reactividad cutánea. Valoración de la tolerabilidad.

Introduction

Rhinoconjunctivitis and allergic asthma are the most prevalent expressions of allergy [1]. The symptoms of both diseases often occur simultaneously, and allergic asthma in particular has become increasingly frequent [2].

House dust mite (HDM) is the most common indoor allergen associated with asthma worldwide [3], and the result of a skin prick test (SPT) with HDM allergens is positive in most asthma patients [4].

Subcutaneous specific immunotherapy (SCIT) has been widely used for decades throughout Europe to increase immunological tolerance and ameliorate symptoms associated with exposure to the causative allergen [5]. SCIT products are efficacious and well tolerated [6,7]. Treatment starts with an updosing phase, in which the patient is given subcutaneous injections with increasing doses of allergen over several weeks. The updosing phase is followed by a maintenance phase, in which the patient is given the same dose of allergen in each subcutaneous injection. The optimization of the allergen to aluminum hydroxide ratio of traditional SCIT products has been investigated with the aim of shortening updosing regimens while maintaining immunogenicity [8].

The new immunologically enhanced SCIT formulation AVANZ Mite mix (ALK-Abelló) is intended to provide the same immunogenicity effect with a simpler and shorter updosing schedule. Therefore, we evaluated the tolerability and the immunological impact of the updosing phase of this new SCIT formulation containing HDM extract.

Material and Methods

Study Design

An open-label, single-arm, phase II/III, clinical trial was conducted at 9 allergy centers in Spain. The study was approved by the corresponding Ethics Committees and by the Spanish Agency for Medicines and Medical Devices, and written informed consent was obtained from all patients before inclusion. The study was carried out in accordance with the Declaration of Helsinki [9] and the International Conference on Harmonization Guideline on Good Clinical Practice [10] and its amendments. The trial was registered at clinicaltrials.gov with identification number NCT01568190.

Patient Population

The inclusion criteria were as follows: age 18-65 years, a clinical history of HDM-induced allergic rhinoconjunctivitis with/without asthma, a positive SPT response to *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae* (wheal diameter ≥3 mm), and positive specific IgE against *D pteronyssinus* or *D farinae* (≥IgE class 2; ≥0.70 kU_A/L) documented within the last 5 years.

The exclusion criteria were as follows: forced expiratory volume in 1 second (FEV_1) <70% of predicted at screening and uncontrolled or severe asthma; clinically relevant history of symptomatic perennial allergic rhinitis and/or conjunctivitis caused by an allergen to which the patient is regularly exposed and sensitized to, except HDM; history of severe asthma exacerbation or emergency visit/admission because of asthma in the previous 12 months; previous treatment with other concomitant allergen immunotherapy or immunotherapy with HDM extracts during the previous 5 years; history of anaphylactic shock due to food, insect venom, exercise, or drug; and history of severe and recurrent angioedema.

Interventions

Patients were treated with AVANZ Mite mix containing equal parts of *D pteronyssinus* and *D farinae* (50:50). Allergen extracts were standardized according to in-house references and expressed in immunologically enhanced standardized quality (SQ+) units.

Patients received 5 weekly updosing injections (300, 600, 3000, 6000, and 15 000 SQ+) of *Dermatophagoides* mix and a maintenance injection (15 000 SQ+) 2 weeks later as part of a short-term single course (approximately 6 weeks) of SCIT. Two days after each visit, patients were contacted by telephone to follow-up on any adverse events (AEs) occurring after each injection.

Blood samples were drawn for immunological determinations at screening and the final visit, and immediate skin test reactivity was assessed using the parallel line assay at the same time points (visits 1 and 6).

Assessments

Tolerability (primary endpoint) was assessed by registering AEs occurring during the 30-minute waiting interval, by

telephone 2 days after each injection, and after the review of diaries issued to patients to record any untoward experiences. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 14.0. Adverse drug reactions (ADRs) were defined as either immediate (0-30 minutes after the injection) or delayed (>30 minutes after the injection). ADRs were also classified as local (injection site) or systemic (generalized signs and/or symptoms distant from the injection site). All local ADRs were recorded regardless of their size and were defined as (diffuse) swelling, erythema, pain, pruritus, injection site urticaria, or injection site reaction (if 2 or more local symptoms occurred). Systemic ADRs were graded from 0 to IV according to the European Academy of Allergy and Clinical Immunology (EAACI) [11].

The immunological effect (secondary endpoint) was assessed by analyzing blood samples to determine specific IgE-blocking factor and specific IgG4 against *D pteronyssinus* and *D farinae* and by performing skin tests to measure the immediate skin response to *Dermatophagoides* mix.

The specific IgE-blocking factor against *D pteronyssinus* and *D farinae* was determined using the ADVIA Centaur immunoassays system (Siemens Medical Solutions Diagnostics). The IgE-blocking factor is a dimensionless number that varies theoretically from 0 (no presence of IgE-blocking components) to 1 (all IgE blocked by binding to allergen). Specific IgG4 was determined using the ImmunoCAP method (Phadia AB).

The change in the immediate skin response (after 15 minutes) to the *Dermatophagoides* mix was measured using the SPT with 3 allergen concentrations (Soluprick *Dermatophagoides* mix 100, 20, and 4 µg/mL) and with histamine and saline solution. Tests were conducted at the same time to avoid variations in the circadian rhythm [12] and in duplicate with a standardized lancet (ALK-Abelló) by experienced personnel. The parallel line assay was used to estimate changes in the skin response, and the results were expressed as the cutaneous tolerance index (CTI).

Statistical Analysis

Statistical analyses were performed on the full analysis set using the available data without imputation of missing values. Tolerability was analyzed using descriptive statistics.

Changes in IgE-blocking factor and IgG4 between visit 1 and visit 6 were compared using the *t* test for paired samples. Statistical significance was set at $P<.05$, and all statistical analyses were performed using SPSS version 17.0 (SPSS Inc).

Results

The trial population comprised 103 patients who were enrolled from January to July 2012. One patient was unable to complete the trial, leaving 102 patients to receive treatment. Of these, 8 discontinued during the trial after having received at least 1 dose of the study treatment (2 due to AEs, 1 lost to follow-up, 2 due to nonadherence to the protocol, and 3 for other reasons) (Figure 1). Baseline characteristics are presented in Table 1. The trial population included 54 men (52.9%) and 48 women (47.1%) with a mean age of 29.3 (7.7) years.

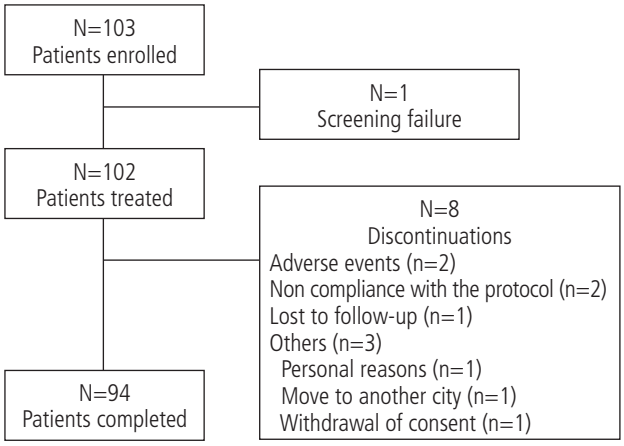


Figure 1. Trial flow diagram.

Table 1. Baseline Characteristics

Characteristic	Value
Age (years), mean (SD)	29.3 (7.7)
Male, No. (%)	54 (52.9)
Ethnic origin, No. (%)	
White	96 (94.1)
Hispanic	2 (2.0)
Asian	2 (2.0)
Other	2 (2.0)
Main concomitant illness, No. (%)	
Conjunctivitis	56 (54.9)
Asthma	49 (48.0)
Allergic rhinitis	44 (43.1)
BMI, kg/m^2, mean (SD)	24.7 (4.6)
Vital signs, mean (SD)	
Systolic blood pressure, mmHg	118.4 (12.9)
Diastolic blood pressure, mmHg	70.4 (8.1)
Heart rate, bpm	73.5 (10.5)
Smoking habits, No. (%)	
Nonsmoker	69 (67.6)
Smoker	22 (21.6)
Previous smoker	11 (10.8)
IgE *D pteronyssinus* CAP class, No. (%)	
Class 0	1 (1.0)
Class 1	0 (0.0)
Class 2	10 (9.8)
Class 3	29 (28.4)
Class 4	31 (30.4)
Class 5	20 (19.6)
Class 6	11 (10.8)
IgE *D farinae* CAP class, No. (%)	
Class 0	1 (1.3)
Class 1	0 (0.0)
Class 2	9 (11.4)
Class 3	28 (35.4)
Class 4	21 (26.6)
Class 5	13 (16.5)
Class 6	7 (8.9)

Abbreviations: BMI, body mass index; bpm, beats per minute.

Table 2. Summary of Adverse Drug Reactions

	No. of Events	No.	(%)
IMP-related adverse events	117	52	(51.0)
Severity			
Mild	116	51	(50.0)
Moderate	1	1	(1.0)
Severe	0	0	(0.0)
Change in treatment schedule			
None	114	50	(49.0)
IMP temporally interrupted	0	0	(0.0)
IMP discontinued	3	2	(2.0)
Prior to first IMP intake	0	0	(0.0
Classification according to EAACI guidelines			
Local reaction	101	48	(47.1)
Systemic reaction	7	5	(4.9)
Grade 0/Nonspecific	9	4	(3.9)
Dose			
300 SQ+	17	13	(12.7)
600 SQ+	8	8	(7.8)
3000 SQ+	30	29	(28.4)
6000 SQ+	27	26	(25.5)
15000 SQ+	30	23	(22.5)
Dose unknown	5	5	(4.9)

Abbreviations: EAACI, European Academy of Allergy and Clinical Immunology; IMP, investigational medicinal product; SQ+, standardized quality units.

Table 3. Nature of Adverse Drug Reactions by System[a]

System organ class/ Preferred term	No. of Events	No. (%)
General disorders and administration site conditions		
Injection site pruritus	21	12 (11.8)
Injection site reaction	47	26 (25.5)
Injection site swelling	26	15 (14.7)
Injection site erythema	2	2 (2.0)
Injection site oedema	2	1 (1.0)
Injection site pain	2	2 (2.0)
Respiratory, thoracic and mediastinal disorders		
Dyspnoea	2	2 (2.0)
Rhinitis allergic	3	3 (2.9)
Nasal obstruction	1	1 (1.0)
Cough	1	1 (1.0)
Musculoskeletal and connective tissue disorders		
Pain in extremity	1	1 (1.0)
Nervous system disorders		
Headache	1	1 (1.0)
Cardiac disorders		
Tachycardia	1	1 (1.0)
Gastrointestinal disorders		
Nausea	1	1 (1.0)
Paresthesia oral	2	1 (1.0)
Oral pruritus	4	1 (1.0)

[a]Coded according to the European Medicines Agency Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases [14].

Tolerability

ADRs are summarized in Table 2. Fifty-two patients (51%) reported 117 ADRs during the trial. All were mild except 1 moderate injection site reaction. In 97.4% of cases, dose adjustments were not necessary, and 98.3% of ADRs had fully resolved at the end of the trial.

A total of 101 local ADRs at the injection site were reported by 48 patients (47.1%). Almost all of the local ADRs were mild—1 was moderate—and did not require modification of the schedule. The patients recovered fully. The most frequent local ADRs (Table 3) were injection site reaction (47 events), followed by diffuse swelling (26 events) and pruritus (21 events). In most cases, treatment was not necessary. Seven systemic ADRs were reported in 5 patients (4.9%). All systemic ADRs were delayed, mild in severity, grade I according to the EAACI classification of systemic reactions, and nonsevere. Systemic ADRs included allergic rhinitis (3 events), dyspnea (2 events), cough (1 event), and nasal obstruction (1 event). Four patients reported 9 nonspecific (grade 0) ADRs. Two patients (2%) withdrew from the study because of AEs: 1 had 2 systemic ADRs (cough and dyspnea), and 1 had a mild ADR, which was classified as a nonlocal, nonsystemic reaction. Half of the systemic ADRs were treated; no patients required adrenaline. In total, 363 adverse events (possibly and unlikely related to the study product) were reported in 79.4% of patients.

Immunological Effects

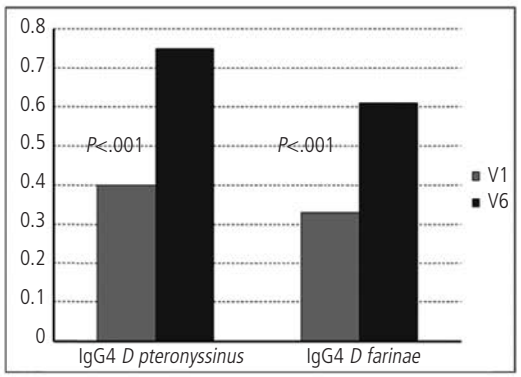
A statistically significant increase in IgG4 and IgE-blocking factor was observed from visit 1 to visit 6 ($P<.001$) (Figure 2).

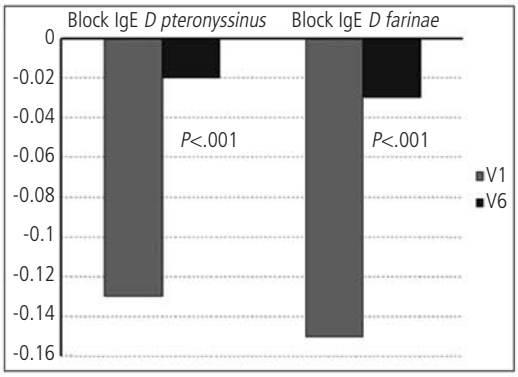
Changes in immediate skin reactivity are shown in Figure 3. Immediate skin reactivity decreased significantly from visit 1 to visit 6 to yield a CTI of 1.44 (95% confidence interval, 1.04-1.98), implying that the dose of *Dermatophagoides* mix would need to be multiplied by 1.44 to reach the same response at visit 6 and ensure a statistically significant difference between the results.

Discussion

We prospectively analyzed the tolerability profile and the immunological effect of a short updosing phase of an immunologically enhanced SCIT formulation based on HDM extract.

As expected, administration of a specific immunotherapy formulation to allergic individuals carries an inherent risk of adverse reactions [13]. All AEs were coded according to the European Medicines Agency Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases [14] using the MedDRA dictionary. Systemic reactions were also classified according to the EAACI Position Paper on Allergen Immunotherapy [11].





	n	Visit 1, mean±SD	Visit 6, mean±SD	Effect size	*P* value*
D pteronyssinus IgG4, mg/L	95	–0.40±0.44	–0.02±0.16	0.35	<.001
D farinae IgG4, mg/L	95	–0.33±0.26	–0.03±0.20	0.28	<.001

	n	Visit 1, mean±SD	Visit 6, mean±SD	Effect size	*P* value*
D pteronyssinus IgE-blocking factor	93	–0.13±0.15	–0.02±0.16	0.11	<.001
D farinae IgE-blocking factor	89	–0.15±0.24	–0.03±0.20	0.12	<.001

Figure 2. Immunological results (IgG4 and IgE-blocking factor) from visit 1 to visit 6.

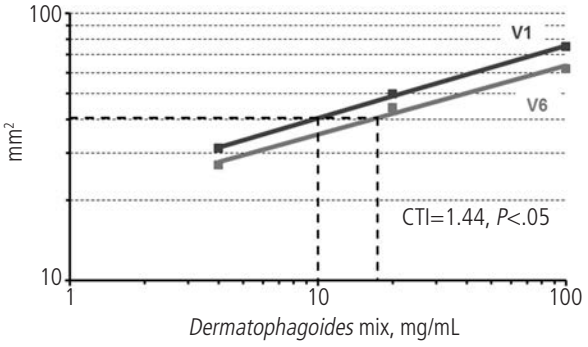


Figure 3. Changes in immediate skin reactivity from visit 1 to visit 6. CTI, cutaneous tolerance index.

A similar trial conducted by Pfaar et al [15] was designed to examine two 5-step updosing schedules (group 1, weekly injections; group 2, injections every 3-4 days) based on the same SCIT formulation but containing 4 sweet vernal grasses and rye administered as a short-term single course (approximately 10 weeks). The results of this investigation showed that 66% of the study population had had at least 1 AE during the study period. Most of the AEs were injection site reactions.

Although around 80% of the population in our trial had at least 1 AE, roughly one-third of the total (363 events) were related to the study drug. Most of these ADRs were local reactions (47.1%). This finding was consistent with those of Pfaar et al [15]. Most of the ADRs were nonsevere or mildly severe injection site reactions and allergic rhinitis, and they resolved spontaneously. The incidence of AEs in our trial was slightly higher than in that of Pfaar et al owing to the different methods used to monitor tolerability: AEs were not only recorded during the 30-minute waiting time after injections and in the follow-up visits as is usually the case, but patients were also asked to record AEs in their patient diary and were contacted by telephone 48 hours after each visit to report any delayed events. Injection site reactions were recorded, coded, and reported regardless of the size of the reaction; other studies only report local reactions (eg, wheal and induration) larger than a given threshold (5-10 cm in diameter) and also included erythema, pain, and pruritus, which are generally not reported.

As for systemic reactions, all 7 (4.9%) systemic ADRs were grade I, mild in severity, nonserious and delayed and led to discontinuation in only 1 case. These findings are better than those recorded by Pfaar et al [15] (27%), who reported that the incidence of systemic reactions was higher and that some reactions were type II.

By contrast, in the prospective study by Tabar et al [16], who administered SCIT based on aluminum hydroxide–adsorbed *D pteronyssinus*, a similar proportion of patients experienced systemic reactions (4.8%), most of which were delayed in onset and asthma-related.

The frequency and severity of adverse reactions vary between studies, and data on tolerability cannot often be compared directly owing to the wide variability in allergen administration schedules, trial design, and safety reporting methodology [6,7,15-18].

Secondary endpoints included analysis of the immune response of the formulation by measuring changes in levels of IgE-blocking factor and IgG4. IgE-blocking factor is induced by immunotherapy [19,20], and IgG4 plays an important role in the mechanism of immunotherapy, as its induction potentially blocks the amount of IgE bound to the allergen, thereby resulting in relief from symptoms [21].

The formulation induced an immune response in the form of statistically significant increases in levels of IgE-blocking factor and IgG4 within 6 weeks of treatment. Our results are consistent with those reported by Pfaar et al [15], who also achieved statistically significant increases in IgE-blocking

factor, IgE, and IgG4 for both group 1 and group 2. Compared with traditional HDM-based SCIT, similar results were obtained in the clinical trial conducted by Tabar et al [18], which was designed to compare the efficacy and safety profile of SCIT based on an aluminum hydroxide–adsorbed *D pteronyssinus* extract given as a 6-week cluster schedule and a conventional 12-week schedule during the updosing phase. During the updosing phases, both schedules induced increases in specific IgE and IgG4 levels, although these appeared quicker in the cluster schedule, simply because the maintenance phase was reached in half the time. Vidal et al [22] conducted a double-blind placebo-controlled clinical trial evaluating aluminum hydroxide–adsorbed *D pteronyssinus*–based SCIT for control of asthma and assessing immunological parameters for *D pteronyssinus*. The authors observed a significant increase in specific IgG4 and in IgE-blocking factor (IgX in the publication) in the active group compared with the placebo group after less than 3 months of treatment.

Quantitative skin tests have been used to estimate changes in the cutaneous response to various concentrations of allergen extracts in a wide variety of applications. The currently recommended reference method in skin tests is the parallel line assay, which is based on the existence of a linear dose-response relationship as a consequence of immunotherapy, as described by Martin et al [23]. In the present study, a reduction in immediate skin reactivity to *Dermatophagoides* mix was achieved within 6 weeks of treatment. This reduction was expressed as the CTI, which is the factor it is necessary to multiply the extract concentration by after SCIT to obtain the same response as at baseline in this linear dose-response relationship. The clinical trials by Tabar et al [18] and Vidal et al [22] revealed similar significant short-term reductions in cutaneous sensitivity to the allergen with the cluster schedules. Although a correlation has been shown between skin test results and symptoms [24], the clinical relevance of the reduction in immediate skin sensitivity warrants further research.

Considering the limited number of studies investigating HDM-based SCIT schedules, our analysis of a new formulation showed that SCIT can be administered with a shorter and simpler updosing phase.

In conclusion, the results of this phase II/III clinical trial showed that the 5-step updosing phase of an immunologically enhanced SCIT formulation of *Dermatophagoides* mix administered in weekly intervals achieved a significant immune response between the start and the end of updosing, a significant reduction in immediate skin reactivity, and a good tolerability profile.

Acknowledgments

The authors would like to acknowledge the AV-M-01 Trial Group for its excellent work, particularly the principal investigators for their contribution to the trial (Isidro Liarte, MD, Luis Ángel Navarro, MD, PhD, Sergia Cruz, MD, PhD, and Teresa Dordal, MD), as well as the participating coinvestigators. We also thank Alicia Marín for managing the trial and Pernille Milvang Grønager and the department of Bioanalytics at ALK (Denmark) for performing the immunological analyses.

We are grateful to Laura R Vallejo for medical writing and editorial assistance on behalf of ALK-Abelló during the journal submission process.

Funding

This trial was sponsored by ALK-Abelló SA. The study investigators received remuneration from ALK for their time spent with each patient and for all trial procedures. The remuneration fulfilled the recommendations of the International Conference on Harmonization Guideline for Good Clinical Practice.

Conflicts of Interest

María Arina is an employee of the Medical Department at ALK-Abelló SA.

Previous Presentation

The study results were presented as a poster at the 2013 SEAIC Congress.

References

1. Ozdoganoglu T, Songu M. The burden of allergic rhinitis and asthma. Ther Adv Respir Dis. 2012;6(1):11-23.
2. Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T. Secular trends of allergic asthma in Danish adults. The Copenhagen Allergy Study. Respir Med. 2001;95(4):258-64.
3. Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the Third International Workshop. J Allergy Clin Immunol. 1997;100(6 Pt 1):S2-24.
4. Terreehorst I, Oosting AJ, Tempels-Pavlica Z, de Monchy JG, Bruijnzeel-Koomen CA, Hak E, van Wijk RG. Prevalence and severity of allergic rhinitis in house dust mite-allergic patients with bronchial asthma or atopic dermatitis. Clin Exp Allergy. 2002;32(8):1160-5.
5. Bousquet J, Van CP, Khaltaev N. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol. 2001;108(5 Suppl):S147-S334.
6. Olaguibel JM, Tabar AI, Garcia BE, Martin S, Rico P. Long-term immunotherapy with an optimal maintenance dose of a standardized *Dermatophagoides pteronyssinus* extract in asthmatic patients. J Investig Allergol Clin Immunol. 1999;9(2):110-6.
7. Tabar AI, Arroabarren E, Echechipia S, Garcia BE, Martin S, Alvarez-Puebla MJ. Three years of specific immunotherapy may be sufficient in house dust mite respiratory allergy. J Allergy Clin Immunol. 2011;127(1):57-63, 63.
8. Rask C, Lund L, Lund G, Urioste S, Thorup S, Bergmann K, Lund K. Optimised allergen: adjuvant ratio potentiates immunogenicity of AlOH-formulated Phleum pratense extract. Allergy. 2010;65(Issue Supplement s92):175.
9. World Medical Association. The Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. Updated 2002. [cited 5-7-2013]. Available from: http://www.fda.gov/ohrms/dockets/dockets/06d0331/06D-0331-EC20-Attach-1.pdf.

10. ICH Harmonised Tripartite Guideline E6 S5. Guideline for Good Clinical Practice. 1996.
11. Alvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E. Standards for practical allergen-specific immunotherapy. Allergy. 2006;61 Suppl 82:1-20.
12. Lee RE, Smolensky MH, Leach CS, McGovern JP. Circadian rhythms in the cutaneous reactivity to histamine and selected antigens, including phase relationship to urinary cortisol excretion. Ann Allergy. 1977;38(4):231-6.
13. Malling HJ, Weeke B. Position Paper: Immunotherapy. Allergy, European Journal of Allergy and Clinical Immunology. 1993;48(Supplement s14):9-35.
14. Committee for Medicinal Products for Human Use. Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases. CHMP/EWP/18504/2006
15. Pfaar O, Jung K, Wolf H, Decot E, Kleine-Tebbe J, Klimek L, Wustenberg E. Immunological effects and tolerability of a new fast updosed immunologically enhanced subcutaneous immunotherapy formulation with optimized allergen/adjuvant ratio. Allergy. 2012;67(5):630-7.
16. Tabar AI, Garcia BE, Rodriguez A, Olaguibel JM, Muro MD, Quirce S. A prospective safety-monitoring study of immunotherapy with biologically standardized extracts. Allergy. 1993;48(6):450-3.
17. Olaguibel JM, Tabar AI, Garcia Figueroa BE, Cortes C. Immunotherapy with standardized extract of *Dermatophagoides pteronyssinus* in bronchial asthma: a dose-titration study. Allergy. 1997;52(2):168-78.
18. Tabar AI, Echechipia S, Garcia BE, Olaguibel JM, Lizaso MT, Gomez B, Aldunate MT, Martin S, Marcotegui F. Double-blind comparative study of cluster and conventional immunotherapy schedules with *Dermatophagoides pteronyssinus*. J Allergy Clin Immunol. 2005;116(1):109-18.
19. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol. 2007;119(4):780-91.
20. Shamji MH, Ljorring C, Francis JN, Calderon MA, Larche M, Kimber I, Frew AJ, Ipsen H, Lund K, Wurtzen PA, Durham SR. Functional rather than immunoreactive levels of IgG4 correlate closely with clinical response to grass pollen immunotherapy. Allergy. 2012;67(2):217-26.
21. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. Curr Opin Allergy Clin Immunol. 2004;4(4):313-8.
22. Vidal C, Tabar AI, Figueroa J, Navarro JA, Sanchez C, Orovitg A, Armisen M, Echechipia S, Joral A, Lizarza S, Lizaso MT, Rodriguez V, de la Torre F. Assessment of short-term changes induced by a *Dermatophagoides pteronyssinus* extract on asthmatic patients. Randomised, double-blind, placebo-controlled trial. Curr Drug Deliv. 2011;8(2):152-8.
23. Martin S, Cuesta P, Rico P, Cortes C. A computer program based on parallel line assay for analysis of skin tests. Allergy. 1997;52(1):97-100.
24. Bousquet J, Maasch H, Martinot B, Hejjaoui A, Wahl R, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. II. Comparison between parameters assessing the efficacy of immunotherapy. J Allergy Clin Immunol. 1988;82(3 Pt 1):439-46.

Manuscript received November 27, 2013; accepted for publication April 23, 2014.

María Arina

Medical Department
ALK-Abelló SA
C/ Miguel Fleta, 19
28037 Madrid, Spain
E-mail: maria.arina@alk.net

