Original Article

Significant Improvement of Specific Bronchial Hyperreactivity in Asthmatic Children After 4 Months of Treatment With a Modified Extract of *Dermatophagoides pteronyssinus*

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Abstract. *Background:* The clinical efficacy of allergen immunotherapy using therapeutic vaccines containing modified allergen extracts has been previously shown.
Objective: To evaluate the clinical efficacy of a vaccine containing depigmented, polymerized extract of *Dermatophagoides pteronyssinus* in asthmatic children, monosensitized to mites, after 4 months of treatment.
Material and Methods: A total of 30 mite-allergic, asthmatic children (age range, 8-16 years) were entered in the study; 15 were treated with the modified allergen extract (active group) and 15 received only pharmacologic treatment (control group). The study was open, controlled and parallel with random allocation of the patients to each of the groups. Efficacy was evaluated using allergen-specific bronchial challenge tests, dose-response skin-prick tests, and symptom and medication scores. The results of the bronchial challenges and dose-response skin-prick tests were compared at baseline and after 4 months of treatment. The build up phase consisted of 4 injections in 2 days, followed by 4 injections of the maintenance dose.
Results: All patients of the active group concluded the study, whereas 2 of the control group did not. In the active group, there was a significant difference in the $PC_{20}FEV_1$ ($P < .01$) after 4 months. The mean allergen quantity needed was 26 μg at baseline vs 309 μg after 4 months (a 12.8-fold increase). There was no difference in the control group (5 μg at baseline vs 8 μg at the end). A significant reduction in the number of cases with dual bronchial response was observed in the treated group ($P < .05$). Two treated patients of this group experienced a negative bronchial challenge after 4 months of treatment. The group of active patients also experienced significant improvement in skin reactivity and symptom and medication scores.
Conclusions: Vaccines containing depigmented polymerized extracts of *D pteronyssinus* are safe and effective in the treatment of mite allergic asthmatic children, and provide clinical benefit after 4 months of treatment.

Key words: Immunotherapy. *Dermatophagoides pteronyssinus*. Asthma. Hyperreactivity. Modified allergens. Allergoids.

Resumen. Se ha demostrado previamente la eficacia clínica de la inmunoterapia con alérgenos utilizando vacunas terapéuticas que contienen extractos alergénicos modificados.
Objetivo: Evaluar la eficacia clínica de una vacuna que contiene extracto despigmentado y polimerizado de *Dermatophagoides pteronyssinus* en niños asmáticos monosensibilizados a los ácaros tras 4 meses de tratamiento.
Material y métodos: Se incluyeron en el estudio un total de 30 niños asmáticos alérgicos a los ácaros (intervalo de edad 8-16); 15 fueron tratados con el extracto alergénico modificado (grupo activo) y 15 sólo recibieron tratamiento farmacológico (grupo de control). El estudio fue de diseño abierto, controlado y con grupos paralelos, con distribución aleatoria de los pacientes a cada uno de los grupos. Se evaluó la eficacia utilizando pruebas de provocación bronquial específicas al alérgeno, pruebas de punción cutáneas para evaluar la relación dosis/respuesta

y puntuaciones de los síntomas y la medicación. Los resultados de las pruebas de provocación bronquial y de punción cutánea para evaluar la relación dosis/respuesta se compararon con los valores basales y tras 4 meses de tratamiento. La fase de iniciación consistió en 4 inyecciones en 2 días, seguidas de 4 inyecciones de la dosis de mantenimiento.
Resultados: Todos los pacientes del grupo activo finalizaron el estudio, mientras que en el grupo de control abandonaron 2 pacientes. En el grupo activo, se observó una diferencia significativa en la PC20 VEF1 ($P < 0,01$) tras 4 meses. La cantidad media de alérgeno necesaria fue de 26 μg al inicio frente a 309 μg tras 4 meses (un incremento de 12,8 veces). No se observó ninguna diferencia en el grupo de control (5 μg al inicio frente a 8 μg al final). Se registró una reducción significativa del número de casos con respuesta bronquial dual en el grupo tratado ($P< 0,05$). Dos pacientes tratados de este grupo experimentaron una provocación bronquial negativa tras 4 meses de tratamiento. El grupo de pacientes activos también experimentó una mejora significativa de la reactividad cutánea y de las puntuaciones de síntomas y medicación.
Conclusiones: Las vacunas con extractos despigmentados y polimerizados de *D. pteronyssinus* son seguras y eficaces en el tratamiento de niños asmáticos alérgicos a los ácaros y proporcionan beneficio clínico tras 4 meses de tratamiento.

Palabras clave: Inmunoterapia. *Dermatophagoides pteronyssinus*. Asma. Hiperreactividad. Alérgenos modificados. Alergoides.

Introduction

Immunotherapy, when introduced during the early phase of an allergic respiratory disease, before irreversible secondary changes occur, may influence the progression of the disorder [1-3].

The clinical efficacy of immunotherapy is dose-dependent [4, 5] and once the maximum dose is reached, the clinical benefit may not be noticed for quite some time [6]. Low doses of allergenic vaccines are inefficient [7], since the effect of a low maintenance dose is comparable to that of placebo. However, the optimal allergen dose to reach the highest clinical efficacy is not completely established and, therefore, the highest tolerated dose is recommended. This dose is defined as one step below the individual dose which may cause an adverse reaction [2]. The use of high doses of well-characterized, standardized, unmodified extracts may produce a higher incidence of unwanted reactions, especially in rush or ultrarush protocols.

Another important issue to be established is the onset of efficacy of allergen immunotherapy. Some studies show that efficacy occurs after few months of treatment [8-10]. Ferrer et al [8] demonstrated a significant difference in the amount of native extract of *D pteronyssinus* needed to produce a drop of 20% in FEV_1 ($P = .0029$) in a group of patients after 6 months of treatment with a depigmented polymerized vaccine containing a combination of *D pteronyssinus* and *Dermatophagoides farinae*. At the end of this study, 10 patients in the immunotherapy treated group vs. 1 in the control group needed more than twice the amount of allergen than at baseline to experience a 20% drop in FEV_1 ($P = .03$). Symptom and medication scores and visual scale improved after 3 and 6 months of treatment only in the active group. A significant decrease in skin test reactivity was also detected in the active group after 6 months, which needed a median of 3 times more allergen to elicit the same reaction as histamine (10 HEP) ($P = .028$), whereas no changes were found in the control group. No serious side reactions were reported. Efficacy has also been demonstrated for modified extracts of *Olea europaea* [11] and *Parietaria judaica* [12] after one year of treatment.

Hunk et al [10] investigated whether IT with *D pteronyssinus* and *D farinae* could decrease airway inflammation as determined by nitric oxide (NO) levels. The authors demonstrated that exhaled NO levels fell significantly after four months of treatment. Peak expiratory flow rates increased significantly and asthma symptom scores decreased after 5 months of treatment. The study by Gardner et al [9] demonstrated that at 9 months, all immunotherapy-treated patients had reduced symptom scores and late-phase cutaneous responses to *D pteronyssinus* compared to baseline levels. The proportions of CD4+ T cells which were IL-10+ increased ($P < .01$), and the proportions of CD4+ and CD8+ T cells which were IL-4+ decreased ($P < .05$), as compared with baseline. These authors concluded that clinically effective subcutaneous immunotherapy with a standardized *D*

Abbreviations used:

PBS:	Phosphate buffer saline
$PC_{20}FEV_1$:	Provocative concentration causing a 20% fall in forced expiratory volume in 1 second.
SBCT:	Specific bronchial challenge test
HEP:	Histamine equivalent prick
LAR:	Late asthmatic response
EAR:	Early asthmatic response
ELISA:	Enzyme Linked Immunosorbent Assay
CI:	Confidence interval
EPR:	Early phase reaction
LPR:	Late phase reaction
NO:	Nitric oxide

pteronyssinus preparation results in decreased numbers of IL-4+ T cells and expansion of CD4+IL-10+ T cells. These findings are consistent with the induction by immunotherapy of T regulatory cells.

The objective of this study was to demonstrate the onset of clinical efficacy after 4 months of treatment and the safety of a depigmented polymerized and aluminium hydroxide-adsorbed *D pteronyssinus* allergen vaccine in pediatric patients suffering from asthma due to sensitization to house dust mite.

Material and Methods

Study Design

The study was designed and conducted as a pragmatic clinical trial, to evaluate how much time was needed for the onset of the expected clinical effect under real-world conditions and to assess the effectiveness of the treatment using objective criteria [13, 14] such as specific bronchial challenge testing. No environmental measures were applied. The study was open, prospective, controlled and parallel with random allocation of the patients to each of the groups. Both groups received medication for their asthma (salbutamol or terbutaline on demand, and bronchial inhaled budesonide) and other medications (loratadine and nasal budesonide). The active group was treated with a modified allergen extract adsorbed onto aluminum hydroxide (Depigoid®), and the control group received only medications. Figure 1 gives an outline of the study design.

Patient Population

Thirty children (19 males and 11 females) with rhinoconjunctivitis and mild/moderate asthma diagnosed according to GINA guidelines [15], due to sensitization to *D pteronyssinus*, were randomly allocated to one of the two groups. The diagnosis was based on the clinical history, positive skin tests to standardized extract of *D pteronyssinus* and negative to other common aeroallergens, detectable specific IgE and positive specific bronchial challenge test (SBCT) to *D pteronyssinus*. The exclusion criteria were those outlined in the WHO position paper on allergen immunotherapy [1]. The active group (administered Depigoid®) consisted of 15 patients (10 males and 5 females, median age 10 years, range 8-15), monosensitized to mites; the control group consisted of 15 mite sensitive, asthmatic individuals (9 males and 6 females, median age 12, range 8-16) who received only pharmacological treatment. For specific challenges, the signed consent of the parents was obtained.

Allergen Vaccine

The modified allergenic extract (Depigoid®) was supplied by Laboratorios LETI, S.L. (Tres Cantos. Spain). The selection of mite species contained in the vaccine was based on a previous study in which we established that *D pteronyssinus* was the main mite species present in mattress dust samples in that area of Spain [16]. The characteristics of the extract used have been published elsewhere [8]. Briefly, a full grown culture of *D pteronyssinus*, with purity greater than 90%, was extracted in 10 mM phosphate buffered saline (PBS) followed by dialysis in 3 kDa flat-bed ultrafiltration membranes (Pellicon, Millipore, Madrid, Spain). The depigmentation step consisted of a controlled mild acid treatment to remove the remaining proportion of adsorbed pigments and produce inactivation of the enzymatic activity. The resulting depigmented allergen preparations were treated with glutaraldehyde [17-19] resulting in a high molecular weight derivative.

The potency of the modified allergenic extracts was measured and adjusted by a reverse phase specific IgE-binding inhibition ELISA system using as standard the in-house reference preparation [20, 21]. The native extract showed a 50% inhibition point of 295 ng and of 18.52 µg (63 times more) for the resulting polymer. The IgG binding activity was measured by means of an ELISA specific IgG inhibition assay [22]. The native extract of *D pteronyssinus* needed 13 ng to produce 50% inhibition of specific human IgG binding to the native extract, and 15 ng for the modified extract.

Der p 1 and Der p 2 allergens were measured in the native and polymerized extracts by means of ELISA using

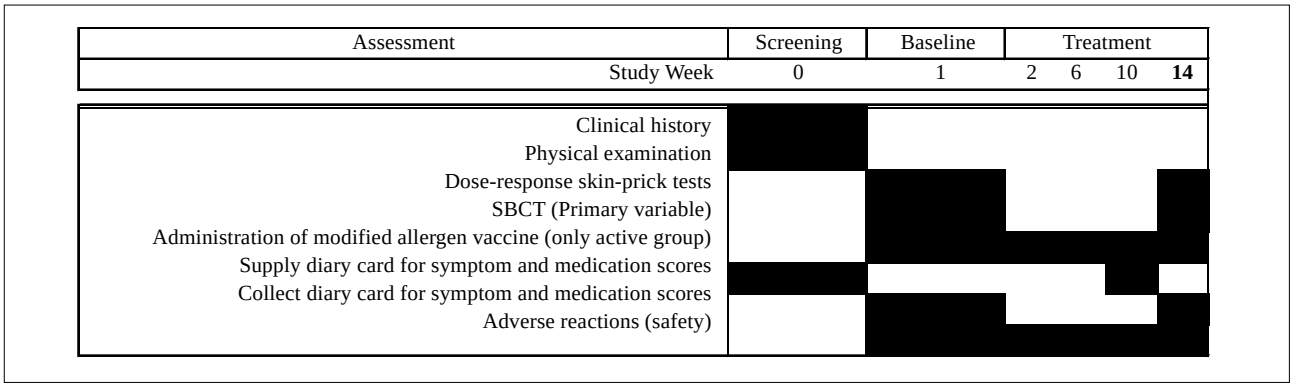

Figure 1. Schedule of the clinical trial.

Table. Schedule of Administration, Dose and Accumulated Dose (Expressed in μg of Freeze-Dried Modified Allergen Vaccine)

Session	Vial	Dose	Day	Modified Allergen Vaccine Administered per Dose μg	Accumulated Modified Allergen Vaccine μg
1	1	0.2 + 0.3	1	4.25	4.25
2	2	0.2 + 0.3	7	42.5	46.75
3	2	0.5	35	42.5	89.25
4	2	0.5	63	42.5	131.75
5	2	0.5	91	42.5	174.25
6	2	0.5	119	42.5	216.75

specific monoclonal antibodies (Indoor Biotechnologies Ltd, Charlottesville, VA, USA). The native extract of *D pteronyssinus* contained 20.35 μg of Der p 1 and 12.3 μg of Der p 2 per mg of freeze-dried extract; Der p 1 and Der p 2 were not detectable in the modified extract. The native extract of *D pteronyssinus* was used for SBCT and dose-response skin-prick tests.

Immunotherapy Schedule

Two vials, numbered as 1 and 2, contained different dilutions of the modified mite extract, and were prepared for each patient. The first day, each patient received 0.2 mL of vial 1, followed by 0.3 mL, 30 minutes later. One week later, 2 more injections of vial 2 were administered in the same manner. Further treatment consisted of 1 injection monthly for 4 months. Each injection of 0.5 mL of vial 2 contained 42.5 μg of depigmented, polymerized extract. The total accumulated dose was 216.75 μg. Table shows the schedule of administration, the dose, and the total accumulated dose.

Safety

Reactions were recorded and classified according to the location (local or systemic), and the time of appearance (immediate, within the first 30 minutes after injection or delayed, when onset occurred after this time). Local reactions were expressed by the length of the longest diameter. Systemic reactions were graded according to EAACI guidelines [2].

Specific Bronchial Challenge Test (SBCT)

The clinical efficacy was evaluate by means of SBCT with the native allergen extract before the start of treatment (baseline) and after 4 months of treatment. Immediate and delayed responses were recorded. The test was performed using native, standardized, unmodified allergen extract of *D pteronyssinus* following the method of Cockcroft et al [23], with slight modifications to adapt to specific allergens [24]. The same batch of native, unmodified allergen extract at 0.002, 0.02, 0.2 and 2 mg/mL (equivalent to 0.1, 1, 10 and 100 HEP/mL) (Laboratorios LETI, S.L. Tres Cantos. Spain) was used throughout the trial. To avoid variations due to storage, it was supplied freeze-dried and vacuum closed to be reconstituted just before use. All patients were tested between 8 a.m. and noon to avoid circadian variations. None of the patients was pre-treated with drugs that could affect the result of the test. The results were expressed as the amount of HEP units needed to achieve a 20% drop in FEV1 ($PC_{20}FEV_1$). Differences between baseline and after 4 months of immunotherapy were analyzed for each patient. The presence of early (EAR) and late (LAR) asthmatic responses was recorded in each patient.

Skin Prick Tests

Patients were skin tested upon admission into the study and after 4 months of treatment. They were done, in duplicate, on the volar surface of the forearm using concentrations of 0.002, 0.02, 0.2 and 2 mg/mL. The vial of maximum concentration had a biological potency of 100 HEP/ml of a standardized *D pteronyssinus* extract (Laboratorios LETI). The same batch of native, unmodified allergen extract was used throughout the trial. To avoid variations due to storage, the extract was supplied freeze-dried and vacuum-closed to be reconstituted just before its use. Histamine HCl 10 mg/ml and a glycerinated saline solution were used as positive and negative controls, respectively. To avoid possible circadian differences in skin reactivity, skin tests were performed between 9:00 AM and 11 AM. None of the patients was pre-treated with drugs that could affect the results of the test. All the reactions were recorded after 15 minutes of application. The area of each wheal was measured by planimetry using a Wacom palette (Wacom Technology Co., Vancouver, WA, USA) and the computer program MacDraft (Microspot, Inc, Boca Raton, FL, USA). The results were expressed as the bioequivalent dose of allergen extract to achieve a wheal of the same size as positive control (10 HEP) [25-27].

Symptom and Medication Scores

All patients recorded daily symptom scores (nose, eyes and lungs) 7 days before each SBCT. Nose (sneeze, blockage, and running), eye (itching, redness, and swelling)

and chest symptoms (breathlessness, wheeze, chest tightness) were scored on a scale ranging from 0 to 3 (0 = none; 1 = slight, the symptom is clearly present but is not troublesome; 2 = moderate, the symptom is present, it is troublesome, but not disabling or insufferable; and 3 = severe, when the symptom is severe, disabling and/or insufferable). The daily total symptom score was calculated as the sum of all individual symptom scores. The intake of medication was recorded on diary cards and was quantified according to Dreborg et al [28] in which the total number of tablets and puffs in 24 hours made up the daily score.

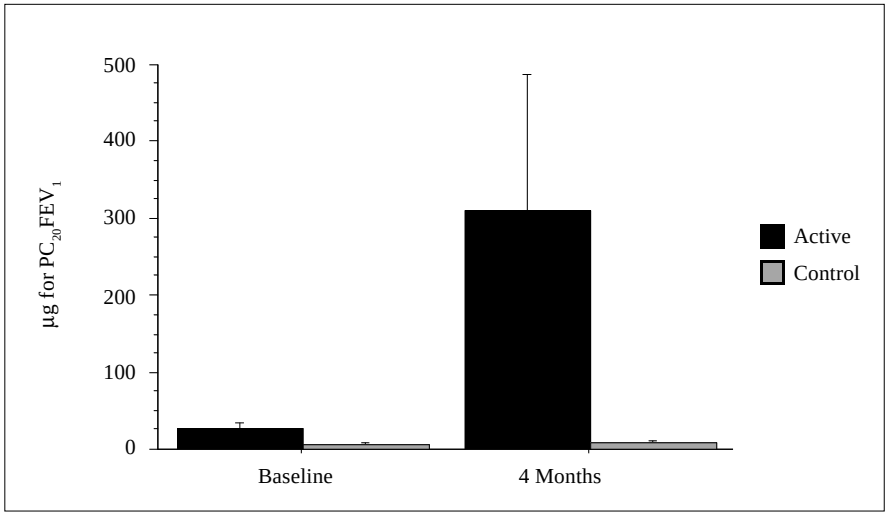

Figure 2. Bar chart of the evolution of the $PC_{20}FEV_1$ of each group (mean ± SE).

Statistical Analysis

Descriptive statistics were expressed as the mean with the 95% confidence intervals, and Wilcoxon and Mann-Whitney tests were used for comparative statistics. The Hodges-Lehmann estimator (with the 95% lower and upper confidence limits) was used to measure the effect size of the differences between the two groups at baseline, and after 4 months of treatment. Contingency table analysis was used to analyze the number of patients that had at baseline and after 4 months a dual EAR and late asthmatic response LAR bronchial response after SBCT. The sample size was calculated by estimating that, at the end of the study**,** 40% of the patients would need more than double the initial amount of allergen extract to experience a positive SBCT in the active group and 10% in the control group [29, 30] for an for an $\alpha = .05$ (two tailed) and a power of 80% (one tailed). The final estimated sample size was 11 patients per group. However, the number of patients was increased by 20% in order to cover the possible drop-outs.

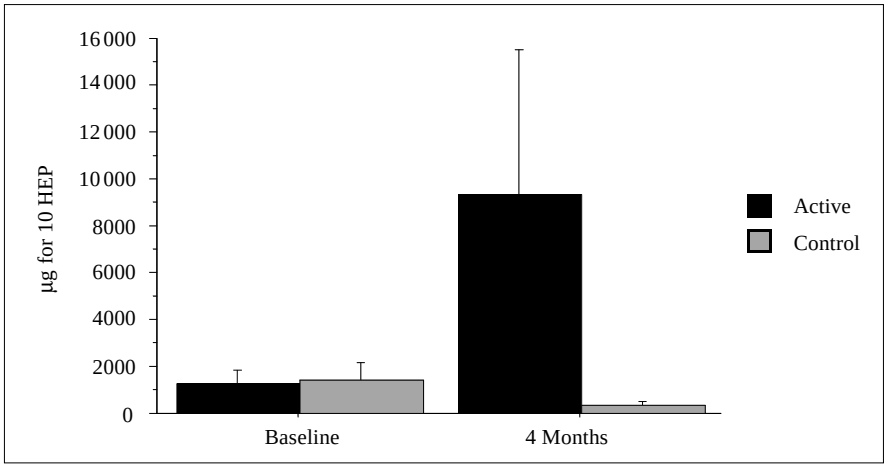

Figure 3. Bar chart of the evolution of the 10 HEP values (mean ± SE).

Results

Patients

Two patients of the control group abandoned the study. The withdrawal of one patient was due to personal decision and the other was due to poor evolution of his asthma.

Specific Bronchial Challenge Tests

All patients had positive responses at baseline. After 4 months of treatment, 2 patients in the active group had a negative bronchial response. At baseline, the mean amount of native allergen needed to induce a drop of 20% in FEV_1 in the active group was 26 µg (95% CI, 9.0 – 43.2), and after 4 months the value was 309.4 µg (95% CI, -39.0 – 657.8; *P*=.0054). The values of the control group were 5.2 µg (95% CI, 2.6 – 7.8) at baseline, and 8.0 µg (95% CI, 2.6 – 13.4; *P*>.05) after 4 months. Figure 2 shows the bar chart (with 95% CI) of the evolution of the values of $PC_{20}FEV_1$ each group.

The results of the Hodges-Lehmann estimator were 5.8 µg (95% CI: -0.2 and 29.6) at baseline and 34.4 µg (95% CI: 5.2 and 95.0) after treatment, confirming that the magnitude of the difference is relevant, since the value 0 is not included in the 95% CI.

When comparing the results between both groups, at baseline the differences were not significant (Mann-

Whitney: p=0.0712), though they became significant after 4 months (Mann-Whitney: p=0.0020).

At baseline in the active group, 13 patients had a dual response (EAR and LAR) after SBCT, while the remaining 2 patients did not. After 4 months of treatment, 8 patients still had a dual response, while 7 did not (p = 0.046). At baseline in the control group, 10 patients had a dual response after SBCT and 5 patients did not; after 4 months of treatment, 9 still had a dual response while 4 did not (p = 0.885).

Skin Prick Test

In the active group at baseline, the 10 HEP value was 1.28 mg (CI 95% 0.25 – 2.35), and after 4 months the value was 9.31 mg (CI 95% -2.79 – 21.41), indicating the need for 7.4 times more native allergen extract to achieve the same result (p = 0.0164). The values of the control group were 1.43 mg (CI 95% -0.03 –2.89) and 0.29 mg (CI 95% -0.08 – 0.67), respectively (p = 0.2860). Figure 3 shows the bar chart (with 95% confidence intervals) of the evolution of the values of 10 HEP in each group.

The results of the Hodges-Lehmann estimator were 0.09 mg (95% CL: -0.1 and 0.55) at baseline versus 0.33 (95% CL: 0.12 and 3.40) at the end. When comparing the results between both groups, at baseline the differences were not significant (p = 0.3345), reaching statistical significance after 4 months (p = 0.0012).

Symptom and Medication Scores

The mean symptom score of the active group at baseline was 1.97 (CI 95% 1.02 – 2.92), and after 4 months the value was 1.00 (CI 95% 0.06 – 1.94) (p = 0.047). In the control group, these values were 2.3 (CI 95% 1.50 – 3.11) and 1.23 (CI 95% 0.14 – 2.31), respectively (p = 0.114).

The mean medication score of the active group at baseline was 1.64 (CI 95% 0.20 – 3.08), and after 4 months the value was 0.47 (CI 95% -0.10 – 1.05) (p = 0.332). The values of the control group were 3.59 (CI 95% 1.72 – 5.45) and 4.87 (CI 95% 1.04 – 8.71), respectively (p = 0.114).

Safety

Each patient received 8 injections in 6 sessions. The total doses administered in the active group were 120. Adverse reactions that occurred in the active group due to the administration of Depigoid were as follows:

Three local reactions occurred in 3 patients:

– Pain and heat over a 24 hour period after the first 2 injections. There was no induration or local inflammation at the site of injection. The reaction was evaluated as mild.

– Pain immediately after the second maintenance dose. There was no induration or local inflammation at the site of injection. The reaction was evaluated as mild.

– Induration (1 cm in diameter) and pruritus after the third maintenance dose. The reaction was evaluated as mild.

Two systemic reactions occurred in 2 patients:

– Dyspnea after the second maintenance dose. The reaction was evaluated as 2 on the EAACI scale.

– Mild exacerbation of asthma and rhinitis after the third maintenance dose. The reaction was evaluated as 2 on the EAACI scale.

Both reactions were considered mild, because no medication (antihistamines, beta 2 agonists, etc.) was needed as treatment. In all cases the administration schedule was not modified and the patients continued with the monthly maintenance dose of 0.5 ml (42.5 µg of modified extract), without additional adverse reactions.

Discussion

Herein we describe the clinical effectiveness and safety of a depigmented, polymerized vaccine of *Dpteronyssinus* in pediatric patients with mild to moderate allergic asthma. The objective outcomes, such as the amount of native, unmodified allergen extract needed to experience a positive bronchial challenge test, and to achieve a skin response of the same size as the histamine control, showed significant differences after 4 months of treatment.

The recommendations outlined in the Nordic Guidelines were followed to demonstrate efficacy [25]. Thus we used the bronchial provocation test as an objective method to document the clinical effect and to calculate the sample size. SBCT has good reproducibility when using FEV_1 as an objective marker of obstruction [31, 32]. It has been shown to be significantly reproducible in a range of one doubling dose (two-fold variation). The reproducibility of allergen SBCT over a period of time grants its reliable use in the clinical evaluation of the efficacy of immunotherapy treatment and in other clinical research studies [33]. The measurement of FEV_1 is an objective outcome that is not affected by suggestion [34]. Placebo was not used following the Note of Clarification on Paragraph 29 added by the World Medical Association Declaration of Helsinki [35], in which it is stated 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 therapy.

The present study suggests that these modified allergen vaccines produce a modification of the natural course of the allergic respiratory disease, which can be seen after 4 months of treatment, as demonstrated by specific bronchial challenge tests. Furthermore, a significant decrease in the number of patients who experienced a dual response after SBCT was seen. Early-phase reactions (EPRs) and late-phase reactions (LPRs) are characteristic features of bronchial asthma, although the pathogenetic mechanisms responsible for each of the responses are not fully defined. EPR appears to depend largely on the release of mediators from airway mast cells, leading to bronchoconstriction and airway edema. The development of the LPR and the concomitant increases in airway reactivity are associated with an influx and activation of inflammatory cells, particularly lymphocytes and

eosinophils in the bronchial mucosa [36-38]. Clinical investigators often use the allergen challenge test to assess new asthma treatments, because attenuation of the LAR has predicted efficacy in subsequent large clinical trials [39].

Few studies have examined the effect of immunotherapy on the changes in frequency and severity of late asthmatic responses, especially in children [40-43]. Allergen-induced pulmonary late-phase responses occur in approximately 40% to 60% of individuals with asthma who inhale a sufficient dose of antigen to cause a 20%, or greater, immediate fall in FEV_1. Our study demonstrates that the late reduction in lung function caused by allergen-inhalation challenge in asthmatic subjects is reversible after the use of immunotherapy with this modified extract, suggesting the anti-inflammatory effect of this treatment. Similar results have been seen by Arvidsson et al. using native birch pollen extracts adsorbed to alum [44]. In this double blind placebo controlled study, a significant increase in allergen dose was required to evoke an early asthmatic reaction in the immunotherapy group (P<.01) after 1 year of treatment. The differences between the active and placebo groups were significant (P<.01). The magnitude of late asthmatic reaction was significantly reduced in the SIT group compared with placebo treated patients (P<.01). Methacholine sensitivity, the number of total eosinophils and eosinophil cationic protein increased significantly in the placebo (P<.02, P<.05 and P<.05 respectively), but not in the active group 24 hours after the last allergen challenge.

Similar results were obtained by Warner et al. in a group of children undergoing immunotherapy with tyrosine adsorbed *D pteronyssinus* extract [45]. In this study, specific immunotherapy was effective in reducing late asthmatic responses, but had only limited effect on reducing immediate or early asthmatic responses. In this study a reduction in late asthmatic response was associated with a greater improvement in asthma symptoms. In our study, 2 patients experienced a negative bronchial challenge after 4 months of treatment, suggesting a significant improvement from baseline.

We decided to use a cut-off point to evaluate efficacy based on a previous study, which showed efficacy after 6 months of treatment. Ferrer et al [8] demonstrated that after six months of treatment an objective improvement in asthma symptoms could be observed in adult patients. At the end of this study, 10 patients in the immunotherapy group vs. 1 in the control group needed more than twice the amount of allergen than at baseline to experience a 20% drop in FEV1 (P = .03). Symptom and medication scores and visual scale evaluation also showed a significant improvement after 3 and 6 months of treatment only in the active group. A significant decrease in skin test reactivity was also detected in the active group after 6 months, which needed a median of 3 times more allergen to elicit the same reaction as histamine (10 HEP) (P = .28), whereas no changes were found in control group. No serious side effects were registered.

It is now established that efficacy in immunotherapy is dose-dependent. In a previous study [46], we evaluated a group of 11 children with a dual response to *D pternonyssinus* upon SBCT. These patients were treated with a standardized, unmodified alum-adsorbed vaccine of *D pteronyssinus*. After 12 months of treatment, 4 children lost the LAR versus 6 after 36 months. The use of modified extracts allows the administration of larger quantities of allergen in a shorter period of time, which could explain the rapid onset of efficacy in the current study.

In our study we have confirmed that allergen immunotherapy using depigmented and polymerized extracts of *D pteronyssinus* is safe and effective in treating children. We have confirmed that efficacy starts early in the treatment process and that it has a significant effect on reducing early and late asthmatic responses.

Acknowledgments

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Competing Interests

There were no conflicts of interests. The investigators were not paid by the laboratories to perform the study. We do not have any commercial ties, or financial interests with the laboratory. There was no direct or indirect influence of the pharmaceutical company on the outcomes of the study.

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