ORIGINAL ARTICLE

Specific Allergen Immunotherapy: Effect on Immunologic Markers and Clinical Parameters in Asthmatic Children

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Abstract

Background: Specific allergen immunotherapy (SIT) is the main treatment modality for achieving long-term symptom relief in perennial allergic diseases.
Objective: The aim of this study was to evaluate the effect of 1 year of house dust mite immunotherapy on the concentrations of 3 immunologic markers: eosinophil cationic protein (ECP), nitric oxide (NO), and monocyte chemoattractant protein 1 (MCP-1). We also compared the effect on asthma symptoms and medication scores, allergen-specific bronchial challenge test, and the skin prick test.
Methods: A total of 31 mite-allergic, asthmatic children (age range, 6-16 years) were enrolled; 19 were treated with SIT and 12 controls who had refused SIT received only drug treatment. Efficacy was evaluated using serum NO, ECP, and MCP-1 levels, and asthma symptom and medication scores, allergen-specific bronchial challenge test, and skin-prick test. The results of the tests were compared at baseline and after 1 year of treatment.
Results: Serum NO and ECP levels decreased significantly in the SIT group ($P = .01$ and $P = .018$) compared to baseline, whereas control group values remained similar. The serum MCP-1 level decreased significantly in both the SIT and control groups ($P = .009$ and $P = .041$, respectively). The SIT group experienced significant improvement in asthma symptoms ($P = .001$) and medication scores ($P = .001$) and skin reactivity to *Dermatophagoides pteronyssinus* ($P = .020$), whereas the control group did not. The results of bronchial challenge to *D pteronyssinus* showed a similar pattern at baseline and after 1 year of treatment in both groups. The tolerated allergen concentration increased in both groups ($P < .05$). Lung function tests, total immunoglobulin (Ig) E and specific IgE to *D pteronyssinus* and *Dermatophagoides farinae* did not change after a year of treatment in either group.
Conclusion: SIT with *D pteronyssinus* improves immunological and clinical parameters in mite-allergic asthmatic children after 1 year of treatment. The skin prick test may be used as a marker of efficacy of therapy.

Key words: Specific allergen immunotherapy. Asthma. House dust mite. Childhood.

Resumen

Antecedentes: La inmunoterapia específica (ITE) es la principal modalidad de tratamiento para lograr la remisión de los síntomas a largo plazo en las enfermedades alérgicas no estacionales.
Objetivo: El propósito del estudio fue evaluar el efecto de la inmunoterapia con ácaros del polvo doméstico durante un año en las concentraciones de tres marcadores inmunológicos: proteína catiónica del eosinófilo (ECP), óxido nítrico (NO) y la proteína 1 quimiotaxina de monocitos (MCP-1). También comparamos el efecto sobre los síntomas del asma y los resultados de la medicación, sobre la prueba de provocación bronquial con alérgenos específicos y la prueba cutánea.
Métodos: Se incluyeron en el estudio un total de 31 niños asmáticos, alérgicos a los ácaros (rango de edad, 6-16 años). A 19 de ellos se les administró la terapia ITE y 12 que rechazaron este tratamiento, siguieron sólo un tratamiento farmacológico. La eficacia se evaluó a partir de los niveles séricos de NO, ECP y MCP-1, los síntomas del asma y los resultados de la medicación, la prueba de provocación bronquial con alérgenos específicos y la prueba cutánea. Se compararon los resultados de las pruebas de la etapa inicial y al cabo de un año del tratamiento.
Resultados: Los niveles de NO sérico y de ECP disminuyeron significativamente en el grupo que siguió el tratamiento ITE ($P = 0,01$ y $P = 0,018$) comparado con la etapa inicial, mientras que los valores del grupo control permanecieron en niveles similares. El nivel de MCP-1 sérico disminuyó significativamente tanto en el grupo ITE como en el grupo control ($P = 0,009$ y $P = 0,041$, respectivamente). El grupo ITE experimentó una mejora significativa en los síntomas del asma ($P = 0,001$) y en los resultados de la medicación ($P = 0,001$), así como en

la reactividad de la piel frente a *Dermatophagoides pteronyssinus* ($P = 0,020$), no ocurriendo así en el grupo control. Los resultados de la provocación bronquial con *D pteronyssinus* manifestaron un patrón similar al basal al cabo de un año del tratamiento en ambos grupos. La concentración de alérgenos tolerada aumentó en ambos grupos ($P < 0,05$). Las pruebas de función respiratoria, la inmunoglobulina (Ig) E total y la IgE específica a *D pteronyssinus y Dermatophagoides farinae* no cambiaron al cabo de un año de tratamiento en ninguno de los dos grupos.
Conclusión: La ITE con *D pteronyssinus* mejora los parámetros clínicos e inmunológicos en los niños asmáticos alérgicos a ácaros al cabo de un año de tratamiento. La prueba cutánea puede usarse como marcador de la eficacia de la terapia.

Palabras clave: Inmunoterapia con alérgenos específicos. Asma. Ácaro del polvo doméstico. Infancia.

Introduction

Specific immunotherapy (SIT) has been used effectively for the treatment of allergic disorders [1-3]. Studies of SIT using standardized extracts have demonstrated improvement in clinical symptoms, medication requirement, lung function, and quality of life [4-6]. SIT may also modify the natural history of allergic diseases, preventing new sensitization and development of asthma in patients with allergic rhinitis [7,8]. SIT seems to modulate the immune response to the causative allergen, inhibiting both early and late responses to allergen exposure [9]. Immunological changes that may contribute to the efficacy of SIT include a decrease in allergen-specific immunoglobulin (Ig) E, an increase in allergen-specific IgG blocking antibodies [10], reduced eosinophil reactivity [11], the generation of allergen-specific suppressor T cells [11], and the deviation of the type 2 helper T cell (T_H2) response in favor of T_H1 response [12].

Asthma is a chronic inflammatory disorder of the airways characterized by inflammation and airway remodeling [13]. The inflammatory process observed in asthmatic patients is the final result of a complex network of interactions between various cell lineages, its mediators, and secreted substances [14]. Many different mediators and cytokines may initiate and partially sustain the inflammation of airways during an asthmatic response [15]. Nitric oxide (NO) is produced by NO synthases in various airway cells and may amplify and perpetuate allergic inflammation [15] and its concentration in exhaled air and sputum is elevated in patients with asthma [16-18]. Eosinophil infiltration is also a characteristic feature of airways in asthma and elevated levels of eosinophils in blood and eosinophil cationic protein (ECP) in serum may be useful markers of airway inflammation in this disease [19-21]. Monocyte chemoattractant protein 1 (MCP-1) might also play a significant role in allergic responses because of its ability to attract monocytes and eosinophils, and to activate mast cells and basophils [22,23].

Most published studies have considered clinical scores as the main parameters, whereas immunological and/or inflammation parameters have been studied only occasionally. The aim of our study was to investigate the efficacy of SIT against *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, the main sources of house dust mite allergens for asthmatic children, on 3 immunologic parameters: ECP, NO, and MCP-1 concentrations. We also compared the relation to asthma symptom and medication scores, allergen-specific bronchial challenge test findings, and skin prick test findings.

Patients and Methods

Thirty-one patients (13 females, 18 males) who were allergic to dust mite as shown by a positive skin prick test and elevated levels of specific IgE to house dust mite were included in this study. The mean (± SD) age of patients was 9.4 ± 2.5 years (range, 6-16 years). All subjects were offered SIT injections. The 19 patients who accepted underwent a 1± year course of SIT injection that was completed in all cases (SIT group) and the remaining 12 subjects who declined SIT (because of individual problems or fear of injections, etc) served as the control group. The study was performed with the approval of the local ethics committee. Informed consent was obtained from all patients.

Asthma was diagnosed according to the guidelines of the Global Initiative for Asthma (GINA) [24]. Both groups were given pharmacotherapy according to GINA guidelines during the study period. All patients had mild asthma and were using inhaled corticosteroids and salbutamol when needed. The study groups were comparable for age, asthma severity, lung function, and bronchial challenge test findings.

Patients were included in the study if they had had asthma symptoms for at least a year and they were sensitized to house dust mite confirmed by a positive skin prick test to the extracts of *D pteronyssinus* and/or *D farinae* and by the presence of specific IgE to *D pteronyssinus* and/or *D farinae* in serum. Exclusion criteria were a) sensitization to other allergens, b) prior immunotherapy, c) respiratory diseases other than asthma, or d) cardiovascular, immunologic, or other severe illnesses.

The clinical efficacy of SIT was assessed by symptom and medication scores [25], and changes in lung function test parameters. Lung function tests, skin prick tests, and a bronchial challenge with *D pteronyssinus* were performed before and after 1 year of SIT. Total serum IgE, specific IgE, ECP, NO, and MCP-1 levels were measured at baseline and after 1 year of SIT.

Skin prick testing was performed with a standardized panel (Allergopharma, Reinbek, Germany) of allergens including grass, tree, and weed pollens, molds, *D pteronyssinus, D farinae,* cat dander, dog dander, cockroach, milk, and

egg white. Histamine dihydrochloride (10 mg/mL) and glycerol diluents were used as positive and negative controls, respectively. After 15 to 20 minutes, wheal size was measured, and the mean diameter was calculated. The sensitivity of the skin test was estimated by the size of the wheal, calculated by the following formula: (DA + dA)/2, where DA is the largest diameter of the wheal, and dA is the midorthogonal diameter of the wheal. A wheal size greater than 3 mm was considered a positive result.

The bronchial challenge test was performed using standardized aqueous extract of *D pteronyssinus* (Allergopharma). The provocation dose concentration causing a decrease of 20% in the forced expiratory volume in 1 second (PD_{20}) was calculated.

A standardized depot mite allergen extract absorbed to aluminum hydroxide (Novo Helisen Depot, Allergopharma) was used for subcutaneous immunotherapy. The activity of extracts of *D pteronyssinus* and *D farinae* is given in standardized quality units expressed as therapeutic units (TU) (concentrations: 5, 50, 500, 5000 TU/mL). The induction phase was performed according to the supplier's recommendations and was followed by a perennial schedule with maintenance injections of 0.8 mL at 4-week intervals all year long according to a the standard procedure followed in our clinic.

Total serum and specific IgE and ECP concentrations were analyzed with the Pharmacia CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden) according to the manufacturer's instructions. A level of specific IgE of 0.35 kU/L or more was considered positive. The serum concentration of NO was measured with the colorimetric method (Nitric Oxide Colorimetric Assay Kit, Calbiochem, EMD Biosciences, Darmstadt, Germany), and MCP-1 was measured with enzyme immunoassay (Neogen Corp, Minneapolis, Minnesota, USA) according to the manufacturer's instructions.

Statistical analyses were performed by means of parametric (the Student *t* test) and nonparametric (the Wilcoxon test for intragroup comparisons and the Mann-Whitney *U* test for intergroup comparisons) tests. A *P*-value was considered statistically significant at less than .05.

Results

All patients completed the study. The 2 groups were comparable in age, sex, and duration of symptoms. SIT was well tolerated and no major local or systemic side effects were reported during the treatment period. The SIT group showed a more marked decrease in asthmatic symptoms and a more marked reduction in drug intake than did the control group.

Mean symptom and medication scores and bronchial provocation test results of both groups are given in Table 1. Both mean symptom and medication scores for asthma decreased significantly in the SIT group in 1 year ($P = .001$) but not in the control group. Results of the bronchial challenge to *D pteronyssinus* were similar at baseline and after 1 year in both groups. The tolerated allergen concentration increased in both groups. PD_{20} was similar in both groups at baseline and at the end of 1 year of treatment. Likewise, baseline lung function parameters were similar to the 1-year test findings in both groups (Table 2). Skin sensitivity to *D pteronyssinus* and *D farinae* decreased significantly after 12 months of SIT (Table 3).

Baseline mean serum NO levels in the SIT and control groups were 1.9 ± 1.2 μM and 1.6 ± 1.4 μM, respectively. Mean serum NO levels decreased significantly in the SIT group after a year ($P = .002$), but no change was detected in the pharmacotherapy group for NO. The baseline ECP level was higher in the SIT group than in the control group and decreased significantly but did not change in the pharmacotherapy (control) group. The baseline serum MCP-1 level in the

Table 1 . Symptom and Medication Scores and Bronchial Provocation Test Results in the Dust-Mite–SIT and Drug Therapy (Non-SIT) Groups*

	SIT Group (n = 19)	Non-SIT Group (n = 12)
Symptom score		
Baseline	2.3 ± 1.1	2.6 ± 0.8
After 1 year	0.9 ± 0.8†,‡	1.6 ± 1.2
Medication score		
Baseline	4.2 ± 2.3	4.2 ± 1.7
After 1 year	1.9 ± 1.3§	2.8 ± 2.4 ‖
Bronchial provocation (tolerated dose), TU		

*SIT indicates specific immunotherapy; TU, therapeutic unit.
†$P = .039$ vs non-SIT group after 1 year
‡$P = .001$ vs baseline of SIT group
§$P = .015$ vs baseline of SIT group
‖$P = .024$ vs baseline of non-SIT group

Table 2. Lung Function Test Results in the Dust-Mite–SIT or Drug Therapy (Non-SIT)*

	SIT Group (n = 19)	Non-SIT Group (n = 12)
FVC, % of predicted		
Baseline	81 ± 19	80 ± 9.1
After 1 year	85 ± 12	88 ± 8.2
FEV_1, % of predicted		
Baseline	92 ± 19	89 ± 12
After 1 year	94 ± 13	96 ± 10
PEF, % of predicted		
Baseline	87 ± 15	95 ± 16
After 1 year	90 ± 10	105 ± 20†
MEF_{25-75}, % of predicted		
Baseline	109 ± 28	107 ± 33
After 1 year	102 ± 13	98 ± 31

*SIT indicates specific immunotherapy; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; PEF, peak expiratory flow; MEF_{25-75}, forced mid-expiratory flow at 25% to 75% of FVC. Data are mean ± SD.
†*P* = .030 vs non-SIT group at baseline.

pharmacotherapy group was higher than in the SIT group and it decreased significantly in both groups (*P* = .003 and *P* = .04, respectively) (Table 4).

Discussion

Our results confirm that SIT is effective for treating asthmatic children sensitized to house dust mite. This study demonstrated that SIT with *D pteronyssinus* and *D farinae* extracts appeared to improve clinical symptoms, and indices of allergic inflammation as determined by serum NO, and ECP concentrations in children who received SIT when compared to the control group. In addition, we also found that unlike the control group, the SIT group showed a tendency for skin reactivity to mite to decrease, although the change did not reach statistically significance.

MCP is a member of the CC chemokine family and is thought to play an important role in the allergic inflammatory process [26]. MCP-1 is chemotactic for monocytes, lymphocytes, and basophils [22,23]. Mechanisms by which MCP-1 may be acting include the stimulation of histamine or leukotriene release from mast cells or basophils and the enhancement of T_H2 polarization [27]. It has been reported that MCP-1 was constitutively present in the airways of asthmatic subjects but its expression and release increased after allergen challenge [5]. In addition, these concentrations of this chemokine have been found to be elevated in patients with atopic dermatitis and allergic rhinitis [28,29]. In addition, it has been demonstrated that MCP-1 neutralization diminishes bronchial hyperresponsiveness and airway inflammation in a murine model [30]. No earlier study has examined the effect of SIT on the serum MCP-1 level. In our study mean levels of serum MCP-1 in the SIT and non-SIT groups were found to be 49.6 and 77.9 pg/mL, respectively. After 1 year of SIT or drug therapy, MCP-1 decreased in both groups. It seems that both SIT and inhaled corticosteroid treatment affect the serum MCP-1 concentration.

NO, which plays an important role in physiologic airway regulation, is synthesized from l-arginine by NO synthase. Three isoforms of NO synthase are known and both the first and the third are constitutively expressed in the human airway, whereas the second is inducible by inflammatory stimuli [15,16]. Exhaled NO has been shown to increase in relation to levels of airway inflammation in asthmatic patients [17,18]. In addition, it has also been reported that asthmatic patients have higher levels of NO in peripheral blood and that serum levels can be used as an additional inflammatory marker in asthma [31]. No study has yet to investigate the effect of SIT on serum NO concentration, although SIT with *D pteronyssinus* and *D farinae* extracts has been found to reduce exhaled NO in asthmatic children with mite allergy and to lead to hyposensitization [32]. However, in one of our studies, we were unable to demonstrate a reduction in exhaled NO in asthmatic patients taking SIT [33]. Contrary to those findings, in this study, serum NO levels decreased after SIT, possibly reflecting a reduction in systemic allergic reaction.

Activated eosinophils release various cytotoxic proteins such as ECP, arachidonic acid metabolites, and oxygen-derived radicals. ECP has been considered to have an important role in the pathogenesis of allergic diseases. Serum levels of ECP have thus been considered useful to monitor airway inflammation in asthma as it correlates with sputum eosinophil counts [21]. Increased ECP release from airway mucosa cells has been said to occur in parallel with raised serum ECP levels [21] and SIT has been shown to decrease serum ECP [21,34]. In this study, serum ECP concentration decreased significantly in the SIT group, but did not change in the pharmacotherapy group, consistent with these previous studies.

In conclusion, we found that house dust mite immunotherapy was effective in improving immunological and clinical

Table 3. Clinical and Laboratory Data Before and After 1 Year of Dust-Mite–Specific Immunotherapy (SIT) or Drug Therapy (Non-SIT)*

	SIT Group (n = 19)	Non-SIT Group (n = 12)
SPT with D pter, mean (range), mm		
Baseline	6 (3-17)	4 (3-6)
After 1 year	3 (1-11.5)†	5 (3-7)
SPT with D pter, mean (range), mm		
Baseline	6 (3-17)	4.5 (3-8)
After 1 year	4 (2-11.5)	6 (4-11)
Total IgE, mean (range), IU/L		
Baseline	368 (37-7713)	276 (87-3400)
After 1 year	400 (18-3640)	234 (32-4100)
Specific D pter IgE RAST class, mean (range), kUa/L		
Baseline	4 (1-6)	4 (1-6)
After 1 year	4 (0-6)	4 (1-6)
Specific D far IgE RAST class, mean (range), kUa/		
Baseline	4 (2-6)	6 (2-6)
After 1 year	5 (2-6)	6 (1-6)
ECP, mean (range), ng/mL		
Baseline	32 (7-73)	23.3 (7-65)
After 1 year	18 (6-81)‡	22 (3-83)
MCP-1, mean (range), pg/mL		
Baseline	49.6 (24.3-899.3)	77.9 (32-652.3)
After 1 year	38.6 (17.6-70.2)§,‖,¶	52.5 (29.6-436.2)†
NO, mean (range), μmol		
Baseline	2.5 (0.5-3.8)	1.6 (0.5-4.5)
After 1 year	0.6 (0.4-3.2)**	1.0 (0.4-2.6)

*SIT indicates specific immunotherapy; SPT, skin prick test; IgE, immunoglobulin E; RAST, radioallergosorbent test; ECP, eosinophil cationic protein; MCP-1, macrophage chemotactic peptide-1; NO, nitric oxide; D pter, *D pteronyssinus*; D far, *D farinae*.
†*P* = .02 vs. SIT group at baseline
‡*P* = .018 vs SIT group at baseline
§*P* = .018 vs non-SIT group after 1 year
‖*P* = .009 vs SIT group at baseline
¶*P* = .041 vs non-SIT group at baseline
***P* = .001 vs SIT group at baseline

parameters in asthmatic children. Our study confirmed an anti-inflammatory effect of SIT evidenced by its decreasing the serum concentration of inflammatory mediators (ECP and NO). Skin prick tests may be used as a marker of efficacy of therapy.

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