Pediatr Allergy Immunol 2007: 18: 508–515
DOI: 10.1111/j.1399-3038.2007.00549.x

PEDIATRIC ALLERGY AND IMMUNOLOGY

Efficacy of long-term sublingual immunotherapy as an adjunct to pharmacotherapy in house dust mite-allergic children with asthma

Ozdemir C, Yazi D, Gocmen I, Yesil O, Aydogan M, Semic-Jusufagic A, Bahceciler NN, Barlan IB. Efficacy of long-term sublingual immunotherapy as an adjunct to pharmacotherapy in house dust mite-allergic children with asthma.
Pediatr Allergy Immunol 2007: 18: 508–515.

Although sublingual immunotherapy (SLIT) is accepted to be a viable alternative of specific-allergen immunotherapy, the efficacy of long-term SLIT in asthmatic children is not well established. The efficacy of 3 yr of SLIT in addition to pharmacotherapy was compared with pharmacotherapy alone in a prospective, open, parallel-group, controlled study. Children with asthma aged 4–16 yr, sensitive to house dust mite (HDM) were followed up for a run-in period of 1 yr and then grouped as those who would receive SLIT + pharmacotherapy (n = 62) or pharmacotherapy alone (n = 28). All patients were evaluated based on symptom-medication scores and lung function tests every 3 months, as well as skin-prick test and serum total immunoglobulin E (IgE) levels annually for 3 yr. Children in the SLIT + pharmacotherapy group demonstrated significantly lower mean daily dose and annual duration of inhaled corticosteroid (ICS) usage when compared with controls. At the end of the 3 yr, within-group comparisons revealed statistically significant decreases in the dose and duration of ICS only in the SLIT group. Furthermore, 52.4% of subjects in the SLIT + pharmacotherapy group were able to discontinue ICS treatment for at least 6 months, which was only 9.1% for the pharmacotherapy group. Three years of SLIT as an adjunct to pharmacotherapy resulted in reduction of both the duration and dose of ICSs and successful discontinuation of ICSs along with improvement in lung functions in HDM-allergic children with asthma.

Cevdet Ozdemir, Didem Yazi, Izlem Gocmen, Ozlem Yesil, Metin Aydogan, Aida Semic-Jusufagic, Nerin N. Bahceciler and Isil B. Barlan
Division of Pediatric Allergy and Immunology, School of Medicine, Marmara University, Istanbul, Turkey

Key words: house dust mite; inhaled corticosteroids; paediatric asthma; sublingual immunotherapy

Cevdet Ozdemir, Seher yildizi sokak Adak Apt. No: 16/10, Etiler 34337, Istanbul, Turkey
Tel.: +90 532 285 3189
Fax: +90 216 326 8030
E-mail: cozdemir@superonline.com

Accepted 13 January 2007

Allergen-specific immunotherapy is the cornerstone in the management of respiratory allergies which targets to modify the immunologic response along with environmental allergen avoidance and pharmacotherapy. Immunotherapy has been successfully administered through the subcutaneous route for decades and shown to be effective in many trials (1). Moreover, it has been shown to be capable of modifying the natural history of allergic diseases, such as preventing the development of asthma in patients with allergic rhinitis (2), as well as development of new sensitizations (3). But variability in safety and clinical efficacy has limited its widespread application because of the disturbance caused by repeated injections to children, which has favoured the development of new alternative routes. Meanwhile, various approaches aimed at

improving the efficacy and safety of subcutaneous immunotherapy (SCIT) have been developed. Many strategies have been adopted in an attempt to standardize practice, including better characterization of the active ingredients through measurement of protein content, determination of the biological activity, estimation of the main allergens that make up the preparation, and the production of pure allergen molecules with the aid of recombinant DNA technology (4–7).

Although the precise mechanism of sublingual immunotherapy (SLIT) is not yet elucidated, there is a growing evidence of regulatory T cells in controlling the development of asthma and allergic diseases. Contact of the allergen with the mucosal Langerhans cells can lead to the capture of the allergen and its transportation to local lymph nodes, which may favor the induction of T lymphocytes that suppress the allergic response, thus inducing immune tolerance by SLIT. Additionally, the production of blocking immunoglobulin G4 (IgG4) antibodies and the involvement of mucosal B cells appear to play a role (8). During the last 15 yr, the sublingual route was extensively studied, and many controlled trials confirmed its efficacy in respiratory allergy. Both clinical trials and post-marketing surveillance studies approved the optimal safety profile in both adults and children (9–21). SLIT is a safe and viable alternative to SCIT and has the convenience of self-administration at home, with a high compliance rate (22). The World Health Organization-Allergic Rhinitis and its Impact on Asthma (ARIA) statement acknowledges that usage of SLIT in children with respiratory allergies is evidence-based (23). On the basis of these results, the most recent recommendation has validated the routine use of SLIT.

In the current study, we evaluated the efficacy of 3 yr of SLIT on clinical and laboratory outcomes as an adjunct to pharmacotherapy in house dust mite (HDM)-allergic asthmatic children and compared it with their peers under pharmacotherapy alone.

Methods

Overall design, patients and inclusion criteria

The study was a prospective, open, parallel-group, controlled study. Children who had been followed up at the Pediatric Allergy and Immunology Unit of Marmara University meeting the inclusion criteria listed below were enrolled in the study: (i) to have a diagnosis of mild–moderate persistent asthma, (ii) monosensitization to HDM (*Dermatophagoides pteronyssinus* and *D. farinae*), (iii) age between 4 and 16 yr, (iv) requirement of inhaled corticosteroids to control the symptoms of asthma, and (v) no previous history of immunotherapy. The diagnosis of mild–moderate persistent asthma was based on Global Initiative for Asthma (GINA) guidelines (24). The Ethics Committee of Marmara University approved the study protocol. Informed consent was obtained from the parents.

After a run-in period of 1 yr, children were divided into: (i) SLIT group, to receive SLIT for 3 yr in addition to pharmacotherapy, and (ii) controls, to receive only pharmacotherapy during that period. At each clinical visit, physical examination and pulmonary function test (PFT) were performed and data on medications were collected from diary cards every 3 months. Additionally, annual skin-prick testing (SPT) and serum IgE-level determination were done (Fig. 1). Measurements obtained at the end of each year of treatment were analysed.

SLIT and concomitant treatments

SLIT. The prescribed SLIT consisted of increasing doses of a standardized extract of 50% *D. pteronyssinus* + 50% *D. farinae* (Stallergenes,

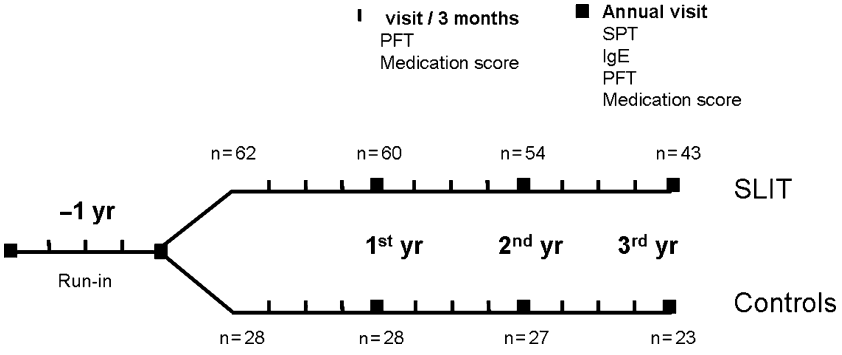

Fig. 1. Study design – asthmatic children allergic to house dust mite under inhaled corticosteroid treatment were followed up for a 1-yr run-in period. Then children were grouped into two as those who would receive SLIT in addition to their ICSs and as those who would not.

Antony Cedex, France). The strength of standardized allergen extract was established by an 'index of reactivity' (IR). The extract was graded in four dilutions: 0.1, 1, 10 and 100 IR/ml. The in-house reference extract (labelled 100 IR) was defined as the concentration eliciting a wheal with a mean diameter of 7 mm by SPT in 30 subjects sensitive to the corresponding allergen. The *D. pteronyssinus* and *D. farinae* contents of 1 ml of 100 IR allergen extract used in this study were 8 and 14 μg, respectively.

Patients received increasing doses from each vial, starting with one drop from 0.1 IR/ml vial, and increasing to 10 drops on day 7. This process was repeated with the 1 IR/ml vial (for days 8–14) and 10 IR/ml vial (for days 15–21). On days 22–28, 1–20 drops were given from the fourth vial (100 IR/ml). Once the 20-drop dose was reached, the patient was switched to maintenance therapy, consisting of 20 drops of 100 IR/ml every day for 4 weeks and then two times a week for the following months. Drops were taken sublingually in the morning before breakfast, kept under tongue for at least 2 min, and then swallowed. At the end of 3 yr of follow-up, an average cumulative dose of 33,000 IR (equivalent to 2.64 mg *D. pteronyssinus*, 4.62 mg *D. farinae*) was administered to each patient in the SLIT group.

Pharmacologic treatment and allergen avoidance. All patients were prescribed appropriate pharmacological treatment and those treatments were monitored according to the protocol of Pediatric Allergy and Clinical Immunology Unit of Marmara University. According to that protocol, for a patient with newly diagnosed asthma an initial dose of 800 μg of budesonide was prescribed and strict allergen avoidance measures were recommended. Patients were then monitored at 2- to 3-month intervals according to the severity and frequency of clinical symptoms, lung functions and environmental avoidance measures. The dose of inhaled corticosteroids (ICS) was then decreased by 50% at each visit until finding the minimal dose which controls the asthmatic symptoms. When the patient was asymptomatic for 3 months with the minimal dose, ICS treatment was discontinued. Treatment with inhaled beta-2 agonists was on an as-needed basis throughout the study period. Both treatment groups were also compared according to the percentage of successful discontinuation of ICS at the end of 3 yr. Successful discontinuation was defined as being asymptomatic after cessation of ICS treatment for at least 6 months of duration.

Patient monitoring

Assessment of treatment and symptoms. Dose of inhaled corticosteroids was adjusted at each clinical visit by the study physician according to the asthma follow-up protocol of our unit. Parents were instructed to fill a diary card during follow-up for daily dose of inhaled corticosteroids.

Pulmonary function tests. Pulmonary function tests were performed by means of computerized spirometry (Zan Flowhandy II; Zan Messgerate GmbH, Oberthulba, Germany) every 3 months during the follow-up visits. The patients used nose clips and were coached through standard forced expiratory manoeuvres. Patients in standing position were asked to take as deep a breath as possible and blow as quick and hard as she or he can into the mouthpiece. The best of three successful manoeuvres was recorded and expressed as the percentage of predicted values for gender, age and height as forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), forced mid-expiratory flow rate (FEF25–75%) and peak expiratory flow rate (PEF). Normal lung function test reference values of Polgar and Promadhat were used to generate the predicted values (25).

Skin-prick test. Skin-prick test was performed annually with 15 common aeroallergens including *D. farinae*, *D. pteronyssinus*, *Alternaria*, *Aspergillus* mix (*A. fumigatus*, *A. nidulans*, *A. niger*), *Cladosporium*, *Penicillium* mix (*P. digitatum*, *P. expansum*, *P. notatum*), *Candida albicans*, Betulaceae, mixture of four cereals (oat, wheat, barley, maize), mixture of 12 grasses (bent grass, Bermuda grass, bromus, cocksfoot, meadow fescue, meadow grass, oat grass, rye-grass, sweet vernal grass, timothy, wild oat, Yorkshire fog), Salicaceae, Compositae, feather mixture (duck, goose, hen), cat hair and dog hair (Stallergens S.A.). Histamine and dihydrochloride saline were used as positive and negative controls. A drop of each allergen extract was introduced via lancets into the skin on the volar side of the left forearm. After 15 min, the wheal reaction was measured as the mean of the longest diameter and the length of the perpendicular line through its middle. A wheal size ≥3 mm was considered positive.

Serum total IgE. Annual serum total IgE levels were measured by Immulite method (Euro/DPC, Llnberis, UK).

Statistical analyses

Statistical analyses were carried out by means of the Statistical Package for the Social Sciences (SPSS) program (Release 11.0; SPSS Inc., Chicago, IL, USA). Differences between groups were tested for significance with chi-squared and *t*-tests. In between-group comparisons, paired-sample *t*-tests were performed. All serum total IgE values were transformed to logarithmic function to have normally skewed data during the analyses. A p-value < 0.05 was considered to be significant.

Results

A total of 90 children (age: mean ± SD 8.3 ± 3.0 yr; female/male 47/43) were enrolled and completed the 1-yr run-in period. Sixty-two children used SLIT in addition to pharmacological treatment, whereas 28 of them were under pharmacological treatment only. There were two drop-outs at the end of the first year, six after the second year and 11 at the end of the third year of follow-up in the SLIT group because of adherence problems and financial reasons resulting from the additional cost of SLIT. Meanwhile, only one dropped out at the end of second year and four at the end of third year in the pharmacotherapy controls. The two groups were similar at enrolment based on demographic, clinical and laboratory parameters, as shown in Table 1.

Corticosteroid-sparing effect of SLIT

Between-group comparison. When compared with controls, annual duration of ICS treatment was significantly less in the SLIT group at the end of the first, second and third years of treatment ($p = 0.048$, 0.001 and 0.003, respectively) (Fig. 2). Additionally, although comparable at baseline, mean daily dose of ICS was significantly less in the SLIT group at the end of the second and third years of treatment ($p = 0.003$ and 0.002, respectively) (Fig. 3). Both treatment groups were also compared according to the percentage of successful discontinuation of ICS at the end of 3 years. Successful discontinuation was defined as being asymptomatic after cessation of ICS treatment for at least 6 months. In that respect, 52.4% of the children in the SLIT group successfully discontinued their ICS medications, whereas this percentage was 9.1% for controls at the end of 3 yr ($p = 0.001$) (Fig. 4).

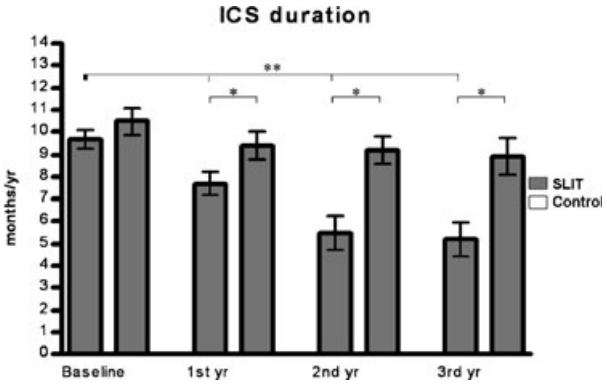

Fig. 2. Annual duration of ICS usage (months/year). *p < 0.05 in between-group comparisons – unpaired *t*-test; **p < 0.05 within-group comparisons – paired-sample *t*-test.

Table 1. Comparison of characteristics of children in SLIT and control groups at baseline

	SLIT (n = 62)	Control (n = 28)	p-value
Gender (female)%	48.4	60.7	0.278*
Age at onset of disease (year)	4.0 ± 3.0	3.3 ± 2.4	0.377‡
Age when our clinical follow-up started (year)	6.3 ± 3.1	6.2 ± 2.2	0.704‡
Age at enrolment (year)	8.8 ± 3.2	7.5 ± 2.4	0.065‡
Run-in period			
Daily ICS dose (budesonide) (μg)	494.98 ± 211	513.07 ± 238	0.753‡
Annual duration of ICS usage (months/year)	9.7 ± 2.9	10.5 ± 2.8	0.276‡
Annual duration of INS usage (months/year)	5.3 ± 4.1	4.6 ± 3.4	0.601‡
# of total attacks	1.3 ± 1.2	0.9 ± 1.4	0.155‡
# of hospitalizations (%)	3.9 ± 0.2	4.3 ± 0.2	0.960*
Diameter of D.Farinae (mm)	4.3 ± 1.7	4.5 ± 1.7	0.612‡
Diameter of D.Pteronyssinus (mm)	5.1 ± 2.5	5.6 ± 2.4	0.395‡
% FVC	101.1 ± 13.9	96.6 ± 15.4	0.302‡
% FEV1	94.1 ± 16.2	95.3 ± 16.3	0.806‡
% FEF25-75	87.3 ± 32.1	91.3 ± 26.6	0.665‡
% PEF	90.9 ± 21.8	81.9 ± 20.8	0.166‡
Serum Total IgE (IU/ml)(mean(range))	737.5 (29–4137)	571.7 (14–1797)	0.276†

*Chi-squared test; †Mann–Whitney *U*-test; ‡unpaired *t*-test.
All values are mean ± standard deviation except those stated otherwise.

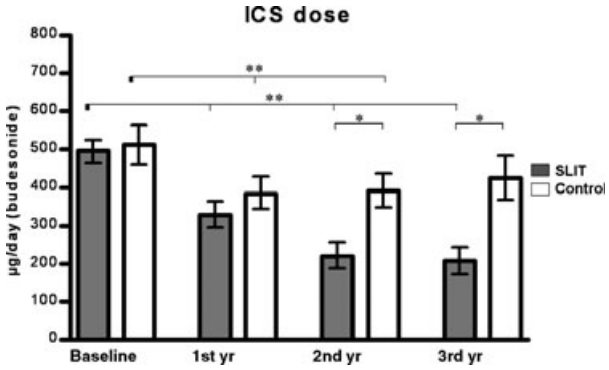

Fig. 3. Mean daily dose of ICS (μg/day (budesonide). *p < 0.05 in between-group comparisons – unpaired *t*-test; **p < 0.05 within-group comparisons – paired-sample t-test.

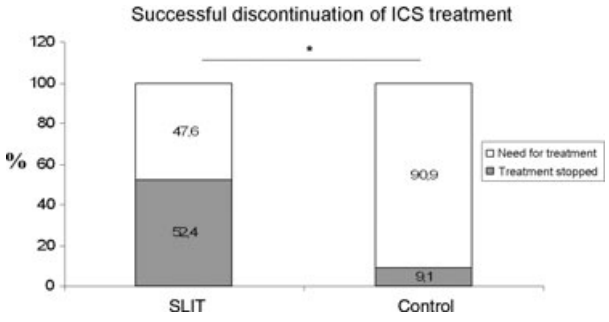

Fig. 4. Discontinuation of corticosteroid treatments for at least 6 months; *statistically significant.

Within-group comparisons. When compared with the run-in period, SLIT resulted in a significant reduction in the annual duration and dose of ICS treatment at the end of the first, second and third years of treatment (ICS duration: $p = 0.002$, 0.0001 and 0.0001; ICS dose: $p = 0.0001$, 0.0001, 0.0001, respectively). Meanwhile, no significant decrease was detected in the annual duration of ICS usage at the end of the study period in the control group, whereas the mean daily dose of that group significantly decreased at the end of the first and second years, but not at the third year (Figs 2 and 3) (ICS duration: $p = 0.231$, 0.264 and 0.085; ICS dose: $p = 0.034$, 0.022 and 0.087, respectively) (Fig. 2).

Pulmonary function tests

Between-group comparisons. At the end of the first year of treatment FEV_1% and FEF25–75% values of the SLIT group were significantly less than controls ($p = 0.003$ and 0.004, respectively). But this difference was not detected at the end of the second and third years of treatment (Fig. 5).

Within-group comparisons. The FEV_1 values of children in the SLIT group demonstrated a significant improvement at the end of the second and third years when compared with baseline ($p = 0.017$ and 0.027, respectively). Moreover, at the end of the third year of SLIT, FEF25–75% values significantly improved ($p = 0.008$). Mean-

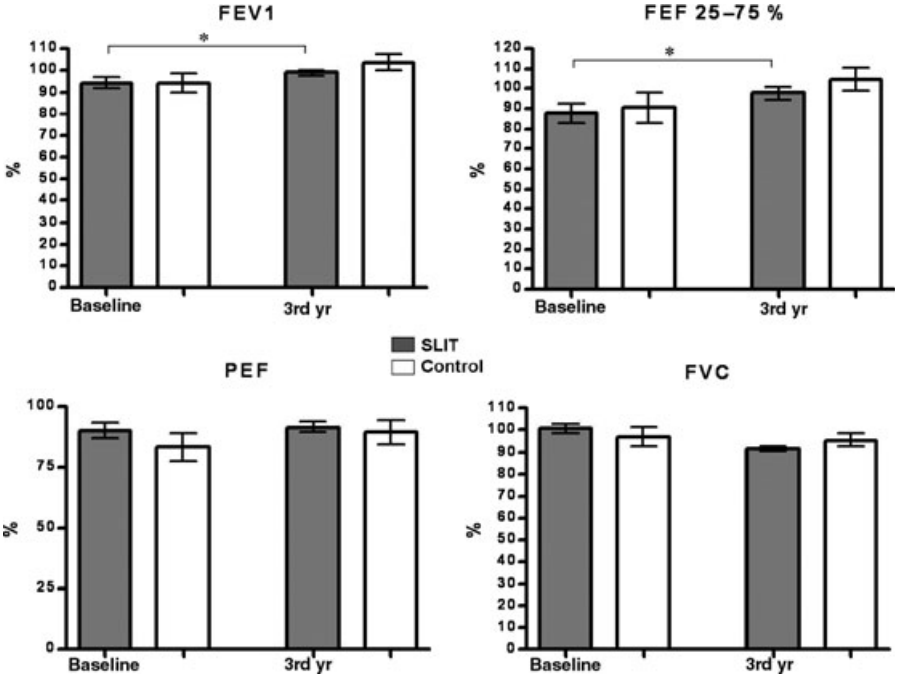

Fig. 5. Comparison of FEV_1, FEF25–75%, PEF and FVC of children in SLIT and control groups at the baseline and at the end of 3 yr of treatment. All the pulmonary function test parameters were comparable at the baseline and at the end of the third year of treatment. Compared with the run-in period, FEV_1 and FEF25–75% were significantly improved in SLIT group ($p < 0.05$).

while, controls did not show any difference (Fig. 4).

SPT reactivity

There was no difference between groups or within groups in the size of skin reactivity to *D. farinae* and *D. pteronyssinus* in either group during the 3 yr of follow-up. Between-group comparisons revealed no significant difference based on new sensitizations.

Serum total IgE

A significant decrease in serum IgE level was detected at the end of the third year in the SLIT group ($p = 0.021$). Controls demonstrated no change in IgE values during the 3-yr of study period (first year: $p = 0.721$; second year: $p = 0.177$; third year; $p = 0.708$). On the other hand, between-group comparisons did not show any significant difference at any time point.

Side effects

No serious adverse reaction was recorded during the follow-up. Mild reactions such as oral itching ($n = 2$), metallic taste sensation ($n = 1$), rhinorhea and sneezing ($n = 1$) did not persist and resolved either spontaneously or by oral antihistamines. These reactions did not lead to discontinuation of SLIT.

Discussion

During the last two decades, SLIT was extensively investigated and many controlled studies confirmed its short-term efficacy and good safety profile in respiratory allergic diseases (9–26). In a meta-analysis of double-blind, placebo-controlled trials that have been carried out in the past decade, Wilson et al. have shown that SLIT is clinically efficacious, although the treatment benefits is about the half that achieved with SCIT in patients with allergic rhinitis (27). On the other hand, Calamita et al. (28), have recently evaluated the clinical efficacy of SLIT for asthma through a systematic review with meta-analysis. They have searched Medline, EMBASE, LILACS and Cochrane library and selected 25 randomized controlled clinical trials with 1706 patients. Their results indicate that SLIT is able to reduce asthma severity significantly when parameter compositions are all analysed by categorical outcomes. They conclude that albeit the magnitude of the effect is not very large, SLIT is found to be beneficial for asthma treatment without causing any severe reactions (28).

On the other hand, little information is available on the possible long-term effect of SLIT in children with asthma. SCIT has been shown to be capable of modifying the natural history of the disease and of preventing the onset of new sensitizations. In a 10-yr prospective study, Di Rienzo et al. demonstrated the long-lasting effect of SLIT in children with asthma due to HDM. In that study, patients receiving SLIT, but not controls demonstrated a significant decrease for the presence of asthmatic symptoms and use of asthma medications 4–5 yr after discontinuation of immunotherapy compared with baseline (29). On the other hand, data on development of new sensitizations for SLIT are still lacking (30).

In the current study, we aimed at determining the steroid-sparing effect of 3 yr of SLIT as an adjunctive treatment to ICS in children with persistent asthma. For this purpose, children receiving regular ICS treatment were followed up for a run-in period of 12 months and then categorized as those who will continue pharmacologic treatment only and those who will receive SLIT in addition to pharmacologic treatment for 3 yr. We acknowledge the fact that a double-blind, placebo-controlled design would have enhanced the power of the study, but it was not feasible for both ethical and practical reasons for that long a period.

At the end of 3 yr, we demonstrated that 52.4% of those children who received SLIT as an adjunct to pharmacologic treatment were able to successfully discontinue their ICS treatment for at least 6 months, whereas only 9.1% of the children who continued to receive only pharmacotherapy were able to do so. In parallel with those findings, annual duration and mean daily dose of ICS significantly decreased in the SLIT group, but not in controls. Moreover, FEV_1% and FEF25–75% values significantly increased at the end of the third year only in the group receiving SLIT.

Previously, in a prospectively designed, open, parallel-group controlled study Di Rienzo et al. (29) treated children with HDM-allergic asthma for 4–5 yr either with SLIT + pharmacological treatment or pharmacological treatment only. Children were evaluated at baseline, at the end of the SLIT course and 4–5 yr after discontinuation based on clinical and lung function parameters, SPT results and IgE measurements. When compared with baseline, there was a significant reduction in the presence of asthma and use of anti-asthma medications only in the SLIT group both at the end of SLIT course and 4–5 yr after discontinuation. Moreover, comparison with

controls revealed significant differences at the two time points. Additionally, PEF values of the SLIT group were significantly higher than controls 4–5 yr after discontinuation. On the other hand, there was no significant difference between groups based on new sensitizations. That study was the first to demonstrate that 4–5 yr of SLIT maintains its efficacy for 4–5 yr after discontinuation in children with asthma allergic to HDM.

Additional support for the long-term efficacy of SLIT came from Marcucci et al. (26) who studied children with allergic rhinitis and/or asthma sensitized to HDM firstly in a double-blind placebo-controlled (DBPC) fashion for 1 yr and subsequently for two further years. Therefore, children initially assigned to placebo had SLIT for 2 yr, whereas those assigned to active treatment had SLIT for 3 yr. Both groups demonstrated significant reduction for rhinitis and asthma scores, but only those treated for 3 yr for drug usage which included scoring for antihistamines, nasal and ocular cromoglycate and beta-2 agonists. Furthermore, nasal eosinophilic cationic protein and tryptase levels following provocation were significantly reduced in the third year, as well as nasal specific IgE levels at the end of the first year in the group treated for 3 yr.

In another 3-yr prospective controlled study, adult patients with rhinitis and asthma monosensitized to birch pollen were randomized to receive either drugs alone or drugs in addition to SLIT and were evaluated in the subsequent four pollen seasons based on symptoms, consumption of medications, nasal smear, eosinophil count and non-specific bronchial hyperresponsiveness. A significant reduction was detected in overall symptoms, bronchodilator use and nasal eosinophils in the SLIT group versus the control group. Additionally, FEV_1%, FEF25–75%, specific airway conductance as well as, non-specific BHR improved significantly starting from the second year only in the active group. That study also confirmed the efficacy of 3 yr of SLIT at both nasal and bronchial level in birch pollinosis (16). In accordance with previously conducted studies, no serious adverse reaction was recorded during our study period. Mild reactions such as oral itching, metallic taste sensation, rhinorhea and sneezing did not end in the discontinuation of treatment.

In conclusion, findings of our study demonstrated that 3 yr of SLIT as an adjunct to pharmacotherapy resulted in reduction of both the duration and dose of ICSs and successful discontinuation of ICSs for at least 6 months along with improvement in lung functions in HDM allergic children with asthma.

Acknowledgments

This study was funded by the Division of Pediatric Allergy and Immunology, Marmara University.

References

1. Bousquet J, Lockey R, Malling HJ. World Health Organization Position Paper. Allergen immunotherapy: therapeutical vaccines for allergic diseases. Allergy 1998: 53 (suppl 50): 13–5.
2. Moller C, Dreborg S, Ferdousi HA, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). J Allergy Clin Immunol 2002: 109: 251–6.
3. Des-Roches A, Paradis L, Menardo JL, Bouges S, Daures JP, Bousquet J. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. VI. Specific immunotherapy prevents the onset of new sensitisations in children. J Allergy Clin Immunol 1997: 99: 450–3.
4. Reid MJ, Lockey RF, Turkeltaub PC, Platt-Mills TAE. Survey of fatalities from skin testing and immunotherapy. J Allergy Clin Immunol 1993: 92: 6–15.
5. Canonica GW, Passalacqua G. Non injectious routes for immunotherapy. J Allergy Clin Immunol 2003: 111: 437–48.
6. Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. Nat Rev Immunol 2006: 6: 761–71.
7. Valenta R. The future of antigen-specific immunotherapy of allergy. Nat Rev Immunol 2002: 2: 446–53.
8. Akdis CA, Barlan IB, Bahceciler N, Akdis M. Immunological mechanisms of sublingual immunotherapy. Allergy 2006: 61 (Suppl 81): 11–4.
9. Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double-blind study. Allergol Immunopathol (Madr) 1990: 18: 277–84.
10. Troise C, Voltolini S, Canessa A, Pecora S, Negrini AC. Sublingual immunotherapy in *Parietaria* pollen-induced rhinitis: a double-blind study. J Investig Allergol Clin Immunol 1995: 5: 25–30.
11. Sabbah A, Hassoun S, Le Sellin J, Andre C, Sicard H. A double-blind, placebo-controlled trial by the sublingual route of immunotherapy with a standardized grass pollen extract. Allergy 1994: 49: 309–13.
12. Clavel R, Bousquet J, Andre C. Clinical efficacy of sublingual-swallow immunotherapy: a double-blind, placebo-controlled trial of a standardized five-grass-pollen extract in rhinitis. Allergy 1998: 53: 493–8.
13. Bahceciler NN, Isik U, Barlan IB, Basaran MB. Efficacy of sublingual immunotherapy in children with asthma and rhinitis: a double-blind, placebo-controlled study. Pediatr Pulmonol 2001: 32: 49–55.
14. Vourdas D, Syrigou E, Potaminaou P, et al. Double-blind, placebo-controlled evaluation of sublingual immunotherapy with standardized olive pollen extract in pediatric patients with allergic rhinoconjunctivitis and mild asthma due to olive pollen sensitization. Allergy 1998: 53: 662–72.
15. Passalacqua G, Albano M, Fregonese L, et al. Randomized controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. Lancet 1998: 351: 629–32.
16. Bousquet J, Scheinmann P, Guinnepain MT, Perrin-Fayolle M, Sauvaget J, Tonnel AB, et al. Sublin-

gual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. Allergy 1999: 54: 249–60.
17. Quirino T, Iemoli E, Sicillani E, Parmiani S, Milazzo F. Sublingual versus injective immunotherapy in grass pollen allergic patients: a double blind (double dummy) study. Clin Exp Allergy 1996: 26: 1253–61.
18. Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G. Clinical, functional, and immunologic effects of sublingual immunotherapy in birch pollinosis: a 3-year randomized controlled study. J Allergy Clin Immunol 2005: 115: 1184–8.
19. Hirsch T, Sahn M, Leupold W. Double-blind placebo-controlled study of sublingual immunotherapy with house dust mite extract (D.pt.) in children. Pediatr Allergy Immunol 1997: 8: 21–7.
20. Ippoliti F, de Santis W, Volterrani A, et al. Immunomodulation during sublingual therapy in allergic children. Pediatr Allergy Immunol 2003: 14: 216–21.
21. Lue KH, Lin YH, Sun HL, Lu KH, Hsieh JC, Chou MC. Clinical and immunologic effects of sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, randomized, placebo-controlled study. Pediatr Allergy Immunol 2006: 17: 408–15.
22. Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G. Randomized controlled open study of sublingual immunotherapy for respiratory allergy in real-life: clinical efficacy and more. Allergy 2004: 59: 1205–10.
23. Wilson DR, Torres LI, Durham SR. Sublingual immunotherapy for allergic rhinitis. (Cochrane review). Cochrane Database Syst Rev 2003: 2: CD002893.
24. National Institutes of Health. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention Revision. National Institutes of Health, National Heart, Lung, and Blood Institute. NIH Publication Number 02-3659, revised 2002.
25. Polgar P, Promadhat V. Pulmonary Testing in Children. Philadelphia, PA: W.B. Saunders 1971: 100–53.
26. Marcucci F, Sensi L, Di Cara G, et al. Three-year follow-up of clinical and inflammation parameters in children monosensitized to mites undergoing sub-lingual immunotherapy. Pediatr Allergy Immunol 2005: 16: 519–26.
27. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. Allergy 2005: 60: 4–12.
28. Calamita Z, Saconato H, Pela AB, Atallah AN. Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method. Allergy 2006: 61: 1162–72.
29. Di Rienzo V, Marcucci F, Puccinelli P, et al. Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: a 10-year prospective study. Clin Exp Allergy 2003: 33: 206–10.
30. Passalacqua G, Canonica GW. Long-lasting clinical efficacy of allergen specific immunotherapy. Allergy 2002: 57: 275–6.