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Original article

House-dust-mite sublingual-swallow immunotherapy (SLIT) in perennial rhinitis: a double-blind, placebo-controlled study

Background: The safety and efficacy of sublingual-swallow immunotherapy (SLIT) in rhinitis caused by house-dust mite were evaluated in a double-blind, placebo-controlled study including 75 patients for 24 months.
Methods: Patients received either placebo or SLIT with a standardized *Dermatophagoides pteronyssinus* (*D.pt.*) – *D. farinae* (*D.f.*) 50/50 extract. The mean cumulative dose was 90 000 IR, equivalent to 2.2 mg of Der p 1 and 1.7 mg of Der f I. Symptom and medication scores were assessed throughout the study. Exposure to house-dust mite, skin sensitivity, and serum specific IgE and IgG4 were assessed before starting treatment and after 12 and 24 months.
Results: Seventy-two patients (36 active–36 placebo) were eligible for intent-to-treat analysis. Thirty-six patients dropped out of the study. The number of patients who dropped out due to lack of efficacy was eight out of 37 (21.6%) in the active treatment group compared to 15 out of 38 (39.5%) in the placebo group (chi-square = 2.81, $P = 0.09$). Total symptom and medication scores decreased significantly after 12 and 24 months ($P < 0.05$) of treatment in both groups, but no significant difference was observed between the active and placebo groups. After 24 months, the number of patients with high levels of indoor allergenic load decreased significantly in both groups compared to baseline data ($P = 0.01$). Specific IgE (*D.pt.* and *D.f.*) increased significantly in the active treatment group after 12 and 24 months, while no change was observed in the placebo group. Specific IgG4 levels were not significantly modified in either group. Two patients in each group reported mild adverse effects. No severe adverse effects were reported.
Conclusions: We conclude that SLIT in rhinitis caused by house-dust mite was safe, but there was a lack of consistent clinical benefit compared to placebo, probably due to the impact of the allergen avoidance measures that lowered the allergen burden.

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Key words: house-dust mite; perennial rhinitis; sublingual immunotherapy.

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Evidence has accumulated over the last decade that exposure to mite allergens plays a critical role in the development of nasal and bronchial hyperreactivity (1, 2). The therapy of perennial rhinitis includes various preventive measures and pharmacologic treatment with or without immunotherapy. Effective elimination of mite allergen seems to be difficult, and even when partial reduction of allergen exposure can be achieved, allergic symptoms may not be affected (3, 4). Immunotherapy by subcutaneous injection of increasing doses of allergens has been part of allergy treatment for 80 years. It has been demonstrated to be a clinically effective treatment for allergic disorders such as rhinoconjunctivitis (5, 6) or asthma (7, 8). As this route of application is inconvenient and occasionally associated with severe systemic adverse effects (9), several trials have been conducted with local (non-injected) allergen-specific immunotherapy by either oral, sublingual, nasal, or bronchial routes. However, the results have been contradictory and the European Academy of Allergology and Clinical Immunology (EAACI) proposed recommendations in 1993 to improve safety and demonstrate greater efficacy (10). Seven well-designed studies demonstrated the clinical effectiveness of sublingual-swallow immunotherapy (SLIT) with grass (11–13), *Parietaria* (14), olive pollen (15), and mite allergens (16, 17). In the light of these data, a recent WHO position paper (18) stated that SLIT may be a viable alternative to parenteral

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injection therapy for treating allergic airways diseases, but further studies are needed to define the indications and therapeutic target dose of this form of immunotherapy.

This study was designed to evaluate the safety and efficacy of SLIT in patients with perennial rhinitis caused by house-dust mite.

Material and methods

Patients

Seventy-five patients (mean age: 26.4 ± 12 years; range: 6–51 years) with a history of perennial rhinitis with or without asthma agreed to take part in this clinical trial. Careful evaluation of clinical history clearly indicated the role of exposure to domestic house-dust mites as the major trigger of the symptoms. Patients also experienced symptoms not sufficiently controlled by antihistamines or topical medications. IgE-mediated rhinitis was demonstrated in all patients selected by positive skin prick tests to *Dermatophagoides pteronyssinus* (*D.pt.*) and *D. farinae* (*D.f.*) standardized extract (Stallergènes SA, Antony, France) and specific IgE (*D.pt.* and *D.f.*) positivity (RAST $\geq$ class 2). Nineteen patients had positive skin tests to one or several other inhalant allergens (cat, dog, *Alternaria*, cockroach, five-grass pollens mix [orchard, meadow, ryegrass, sweet vernal, and timothy], *Artemisia* pollen, ragweed pollen, and birch pollen), but none of these skin sensitivities were associated with evidence of clinical sensitization. Patients sensitized to cat or dog allergen and living with pets at home were not included in the study. None of the patients had previously received immunotherapy with *D.pt.*, none used oral or inhaled steroids, and none had any other significant illness. The study protocol was approved by the ethics committee of the Pellegrin Children's Hospital, Bordeaux, France, and patients, or both parents of minors, signed informed consent forms before starting the study.

Allergen preparation

The standardized extract used throughout the study was the same batch of equal proportions of a mixture of *D.pt.* and *D.f.* (Stallergènes SA, Antony, France). The extract was graded into four concentrations: 1, 10, 100, and 300 IR/ml. The in-house reference extract (labeled 100 IR [index of reactivity] is defined as the concentration eliciting by skin prick test a geometric mean wheal size of 7 mm diameter in 30 subjects sensitive to the corresponding allergen. The Der p 1 and Der f 1 contents of 1 ml of the 100 IR allergen extract used in this study were 4.8 and 3.7 μg, respectively.

Immunotherapy

Patient selection was not followed by a run-in observation period. Subjects were randomized to receive either a *D.pt./D.f.* preparation in 50% aqueous glycerol or placebo. The placebo preparation was identical to active treatment in terms of composition, appearance, presentation, taste, and color, but obviously did not contain allergens. The duration of treatment was 24 months, and treatment was initiated between November 1994 and January 1995. The study was completed in January 1997. During a 4-week incremental dose period, patients took daily increasing doses from 1–10 drops of the 1-IR/ml vial from day 1 to day 4, then 1–10 drops of the 10-IR/ml vial from day 5 to day 8, then 1–20 drops of the 100-IR/ml vial from day 9 to day 15, and finally 5–20 drops of the 300-IR/ml vial during the next 2 weeks. The highest dose (20 drops from the 300-IR/ml vial) was administered daily for 4 weeks, and then 3 days per week throughout the maintenance phase. Each dose had to be taken in the morning before breakfast, and drops of allergen had to be kept under the tongue for at least 2 min before swallowing. According to this schedule, an average cumulative dose of 90 000 IR (equivalent to 2.2 mg of Der p 1 and 1.7 mg of Der f 1) of allergens was administered to each patient undergoing active treatment, at the end of the trial.

Skin reactivity

Before treatment, after 12 months, and at the end of the trial, quantitative skin prick tests were performed with a Stallerpoint® device, with five serial threefold dilutions of *D.pt.* and *D.f.* extracts (300, 100, 30, 10, and 1 IR/ml, Stallergènes SA). The wheals were marked after 15 min with a thin pen and then transferred onto a transparent adhesive tape.

Symptom and medication diary

Patients (or their parents) were instructed to keep a diary during the treatment period, for weekly evaluation of symptoms according to a 4-point scoring system (0: no symptoms; 3: severe symptoms) for each nasal symptom (sneezing, nasal discharge, itching, and nasal obstruction). The maximal cumulated clinical score per patient was 12. Although some patients had ocular symptoms associated with rhinitis, only nasal symptoms were evaluated for analysis of efficacy. When necessary, patients were allowed to use the following drugs: antihistamine (cetirizine 10-mg tablets), intranasal corticosteroid (flunisolide), and betamethasone 0.5-mg tablets. Other medications, such as long-lasting antihistamines, cromones, and parenteral corticosteroids, were not permitted during the study. The patients had to record on the same diary card whenever they used medications, and a specific weekly score was

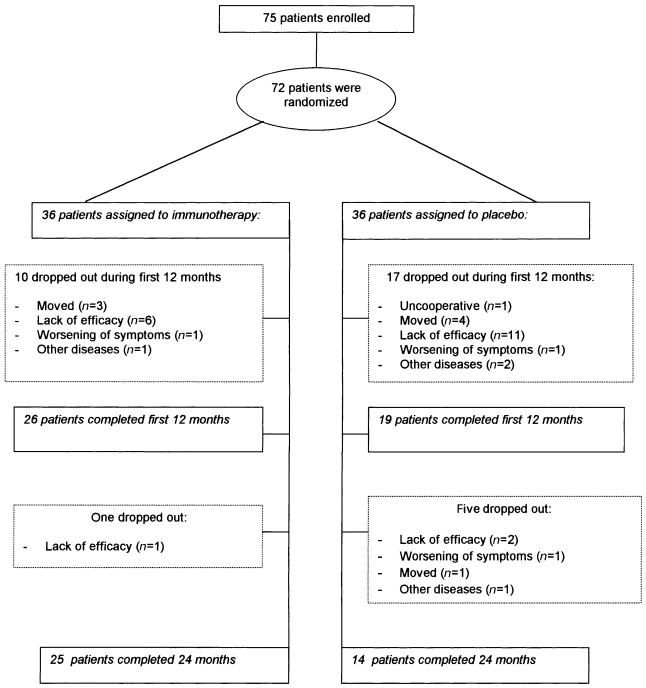

Figure 1. Trial profile.

assigned (1 point: one puff of nasal flunisolide per nostril, 2 points: one cetirizine tablet, 3 points: one betamethasone tablet). This assigned score was established not on the basis of the strength of each drug, but rather on the frequency of use, as, in this study, nasal corticosteroids were allowed to be used more frequently than antihistamines as first-line treatment.

Patients were also asked to assess subjectively their symptoms according to a 4-point scoring system (1: totally improved, 2: partially improved, 3: unchanged, and 4: worsened) and on a 100-mm visual analog scale (VAS) (from 0 mm [no improvement] to 100 mm [remarkable improvement]. The physicians also expressed their opinion of each patient's clinical status (1: totally improved, 2: partially improved, 3: unchanged, and 4: worse).

Immunologic parameters

D.pt.- and *D.f.*-specific IgE and IgG4 were determined at the end of the trial by commercial kits, according to the manufacturer's instructions (CAP System EIA method, Pharmacia AB, Uppsala, Sweden), on serum samples taken at the beginning of the study, and after 12 and 24 months. IgE values were expressed as kU/l, and IgG4 values were expressed as a percentage of a reference serum standard.

Exposure to house-dust mite

Patients were asked to vacuum-clean their bedroom and mattresses on enrolment in the study, and then at 12 and 24 months. They had to use their own vacuum cleaner at a constant vacuuming time of 2 min/m^2. Dust samples were stored at 4°C in sealed vacuum paper bags until analysis. Samples were processed without sieving. The concentration of house-dust mite was determined by a semiquantitative guanine assay (Acarex Test®) (Allergopharma, Reinbek, Germany). Results were expressed on a 4-point scale: 0: guanine content of <0.6 μg/g of dust, 1: 0.6–2.5 μg/g, 2: 2.5–10 μg/g, and 3: >10 μg/g (19). Before starting treatment, all patients were asked not to use specific measures to avoid house-dust mites during the study, in order to maintain the same level of exposure to house-dust mites.

Table 1. Clinical data of patients

	Treatment	
	Active	Placebo
Number of patients	36	36
Sex (M/F)	14/22	15/21
Age		
Mean age (years) ± SD	29.6 ± 12.4	22.7 ± 10.7*
Range	12–51	6–47
Mean duration of rhinitis (years)	9.6 ± 6.6	8.2 ± 8.2
D.pt.[1] skin prick test (mean wheal ± SD)	8.6 ± 4.7	7.2 ± 3.3
D.f.[2] skin prick test (mean wheal ± SD)	7.5 ± 3.6	6.6 ± 3.4
Specific IgE *D.pt.* (kIU/l)	25.3 ± 28.4	37 ± 33.6
Specific IgE *D.f.* (kIU/l)	18.8 ± 27.5	31.0 ± 32.3*

[1]*D. pteronyssinus.*
[2]*D. farinae.*
*$P<0.05$, comparison between placebo group and active group.

Statistical analysis

Qualitative data were analyzed by the chi-square test or Fisher's exact test. Symptoms, medication intake, skin tests, and IgE and IgG4 specific to house-dust mite were analyzed statistically by nonparametric tests; the Wilcoxon rank sum test was used for intragroup analysis, and the Mann–Whitney U-test for intergroup analysis. All tests were two-tailed, and the level of significance was set at 0.05.

Results

Seventy-five patients were included in this study. Seventy-two patients were available for intent-to-treat

Table 2. Change in exposure to house-dust mite (mattress samples)

	0 months		12 months		24 months	
Acarex class (μg/g dust)	Active ($n=35$)	Placebo ($n=33$)	Active ($n=29$)	Placebo ($n=26$)	Active ($n=22$)	Placebo ($n=13$)
Class 0 (<600)	0	0	0	0	1	0
Class 1 (600–2500)	11	10	14	9	15	9
Class 2 (2500–10 000)	15	16	14	16	5	4
Class 3 ($>10\,000$)	9	7	1	1	1	0

analysis (36 in the active group and 36 in the placebo group). Three patients were excluded from the intent-to-treat analysis due to missing data for the primary end point. Twenty-seven patients dropped out of the study during the first year, and another six patients dropped out during the second year (Fig. 1). The number of dropouts was significantly greater in the placebo group than in the active group (22 patients and 11 patients, respectively; $P<0.01$). The number of patients who dropped out because of lack of efficacy was eight out of 37 (21.6%) in the active treatment group compared to 15 out of 38 (39.5%) in the placebo group (chi-square = 2.81, $P=0.09$). Other reasons for discontinuing immunotherapy were poor compliance and loss to follow-up for nine patients (three in the active group; six in the placebo group) and other diseases for four patients (one in the active group; three in the placebo group).

The baseline clinical and demographic characteristics of the 72 patients eligible for analysis (Table 1) did not reveal any significant differences between the active treatment and placebo groups except for mean age and *D.f.*-specific IgE levels.

Exposure to house-dust mite

The results were available for 68 patients on inclusion and showed that 47 out of 68 patients (70%) had class 2–3 guanine levels in their mattress samples. The guanine levels were not significantly different between the active and placebo groups (Table 2). After 24 months, the number of patients with class 2–3 guanine levels decreased significantly in both groups ($P=0.01$), while there was a concomitant increase in the number of patients with low guanine levels (class 1), indicating a marked change of indoor allergen load.

Symptom scores

The patients' clinical status was assessed 1 month after starting treatment due to the lack of run-in period to assess the baseline clinical score. Symptom scores were not significantly different between the two groups 1 month after starting treatment (Table 3). A statistically significant decrease in weekly nasal symptom scores, except for nasal itching, was observed in both groups after 12 months of treatment ($P<0.05$). The total symptom score decreased by 31% in the active group and 17% in the placebo group, with no significant difference between the two groups. After 24 months, all symptoms had decreased significantly ($P<0.05$) in the active and placebo groups, except for the nasal itching score in the placebo group. When compared to the 1-month score, the total symptom score was reduced by 40% in the active group and 20% in the placebo group, but the between-group difference was not significant. As indicated in Fig. 2, for the 42 patients who completed the trial, the total symptom scores decreased in a similar way in the two groups over the entire treatment period, and the difference was not significant. Nor did the patients' and physicians' subjective assessments by VAS or the 0–4 scale reveal any significant difference between active treatment or placebo (data not shown).

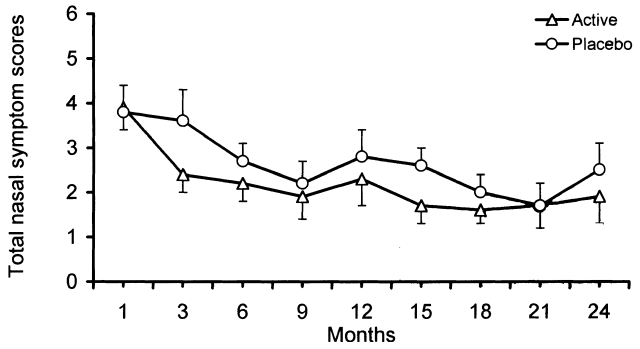

Figure 2. Mean (±SEM) total nasal symptom scores in active treatment ($n=24$) and placebo ($n=18$) groups of patients who completed trial.

Medication scores

After 12 and 24 months of treatment, the score of nasal corticosteroid and H_1-antihistamine intake was significantly reduced in the active group, while only the nasal

Table 3. Intragroup analysis of nasal symptom scores (mean weekly scores ±SD)

	Active ($n=36$)			Placebo ($n=36$)		
	1 month	12 months	24 months	1 month	12 months	24 months
Sneezing	1.0±0.5	0.7±0.4*	0.6±0.5§	1.1±0.6	0.9±0.5*	0.8±0.6§
Rhinorrhea	1.0±0.5	0.7±0.3*	0.6±0.5§	1.1±0.7	0.9±0.5*	0.8±0.6§
Nasal itching	0.6±0.6	0.5±0.4	0.4±0.5§	0.7±0.6	0.6±0.5	0.6±0.6
Nasal blockage	1.0±0.7	0.7±0.5*	0.6±0.5§	1.0±0.7	0.8±0.6*	0.8±0.6§
Total symptom score	3.8±1.7	2.6±1.5*	2.3±1.9§	4.0±1.9	3.3±1.9*	3.2±2.4§
Number of weeks without symptoms	0.3±0.8	23.8±28.0	34.3±34.9#	0.5±1.1	19.8±28.3	27.5±39.9

*Level of significance for intragroup differences between 1 month and 12 months: $P<0.05$ (Wilcoxon paired T-test).
§Level of significance for intragroup differences between 1 month and 24 months: $P<0.05$ (Wilcoxon paired T-test).
#$P<0.05$, 24 months *vs* 12 months.

Table 4. Intragroup analysis of medication scores (mean weekly weighted scores ± SD)

	Active ($n=36$)			Placebo ($n=36$)		
	1 month	12 months	24 months	1 month	12 months	24 months
Flunisolide	2.7 ± 4.1	0.7 ± 1.0*	0.5 ± 0.9§	2.5 ± 3.5	1.0 ± 1.9*	0.8 ± 1.9§
Cetirizine	5.5 ± 5.6	3.1 ± 3.5*	2.3 ± 3.7§	5.9 ± 5.6	4.3 ± 4.5	4.5 ± 5.3
Betamethasone	1.06 ± 3.7	0.8 ± 2.0	1.3 ± 3.9	1.8 ± 9.0	1.0 ± 1.8	0.7 ± 1.7
Weekly medication score	9.2 ± 10.6	4.6 ± 4.2*	4.1 ± 5.5§	10.2 ± 11.4	6.3 ± 6.1*	6.1 ± 6.8§

*Level of significance for intragroup differences between 1 month and 12 months: $P<0.05$ (Wilcoxon paired T-test).
§Level of significance for intragroup differences between 1 month and 24 months: $P<0.05$ (Wilcoxon paired T-test).

corticosteroid score was significantly reduced in the placebo group (Table 4). The total medication score decreased significantly after 12 ($P<0.05$) and 24 months ($P<0.05$) of treatment in both groups, and the between-group difference was not significant.

A cumulative symptoms plus medication (Table 5) score was calculated and showed a significant decrease after 12 and 24 months of treatment in the active group (50% reduction) and placebo group (35% reduction), but no significant differences between the two groups.

Skin reactivity

No significant decrease of wheal areas obtained by prick test with *D.pt.* was observed for either active treatment or placebo groups (data not shown).

Immunologic parameters

As indicated in Table 6, for the 42 patients who completed the trial, specific *D.pt.* and *D.f.* IgE levels increased significantly after 12 months of treatment in the active treatment group ($P<0.05$), but remained unchanged in the placebo group. After 24 months, specific IgE decreased slightly in the active group, while the IgE levels in the placebo group were lower than those obtained at enrolment.

Specific IgG4 did not change significantly in either the active group or the placebo group at any of the follow-up investigations (Table 6).

Safety assessment

Two patients receiving active treatment reported local adverse reactions (mouth itching and burning). In the placebo group, one patient reported an episode of mild asthma, and another one had an episode of rhinosinusitis. No severe adverse effects were reported or detected.

Discussion

This study compared house-dust-mite SLIT and placebo, administered for 24 months, for perennial allergic rhinitis. One feature of this study is the large number of patients who dropped out, a factor which decreased the power of the analysis. However, it should be stressed that the fact that a large number of patients dropped out because of lack of efficacy in the placebo group indicates a trend toward better compliance in the active treatment group due to the efficacy of immunotherapy. A significant reduction of nasal symptoms and medication scores was observed in the active and placebo groups.

According to the criteria defined by Malling (20), the efficacy of immunotherapy in double-blind, placebo-controlled trials must be supported by an improvement of symptom/medication scores by more than 30%, and this was the case in both groups of this study with no significant differences. The clinical efficacy of immunotherapy in rhinitis caused by house-dust mite has been insufficiently documented, and only two studies, performed by subcutaneous injections, satisfied these criteria (21, 22). The failure to demonstrate a significant difference between the immunotherapy group and the placebo group in our study was probably due to the insufficient doses administered to the patients. SLIT trials performed in rhinitis caused by house-dust mite have reported discordant results. In a previous study, Tari et al. (16) demonstrated clinical improvement of nasal and bronchial reactivity after 18 months of SLIT in patients with rhinitis and asthma. However, the authors did not indicate the cumulative dose of major allergen Der p 1 administered to the patients.

Table 5. Cumulated symptoms-medication score (mean weekly scores ± SD)

	Active ($n=36$)			Placebo ($n=36$)		
	1 month	12 months	24 months	1 month	12 months	24 months
Symptoms-medication score	13.0 ± 11.5	7.2 ± 1.0*	6.4 ± 6.3§	14.3 ± 11.6	9.5 ± 6.8*	9.2 ± 8.0§

*Level of significance for intragroup differences between 1 month and 12 months: $P<0.01$ (Wilcoxon paired T-test).
§Level of significance for intragroup differences between 1 month and 24 months: $P<0.01$ (Wilcoxon paired T-test).

Table 6. Specific IgE (kU/l) and specific IgG4 (%) changes (mean ± SD)

	Active ($n=24$)			Placebo ($n=18$)		
Months	0	12	24	0	12	24
IgE *D.pt.*	44.3 ± 63.2	56.7 ± 63.3*	48.2 ± 59.5	54.9 ± 68.3	54.3 ± 74.2	40.5 ± 52.3
IgE *D.f.*	28.4 ± 45.8	7.3 ± 56.8§	41.3 ± 77.9	41.3 ± 66.5	33.4 ± 61.5	
IgG4 *D.pt.*	4.5 ± 1.7	5 ± 1.6	4.8 ± 1.8	4.8 ± 1.7	4.8 ± 1.7	5 ± 2.3
IgG4 *D.f.*	4.8 ± 1.1	4.1 ± 1.2	4.2 ± 1.4	4.2 ± 1.4	4 ± 1.4	4 ± 1

Level of significance for intragroup differences: *$P \leq 0.05$ (12 months *vs* 0 month); §$P \leq 0.05$ (24 months *vs* 0 month).

In another study using a chemically modified allergen (allergoid) of house-dust mite, Passalacqua et al. (17) showed a significant reduction in nasal symptom scores in the immunotherapy group compared to the placebo group after 24 months of treatment. As stated by the authors, modification of the allergen into an allergoid prevented determination of the level of Der p 1 in the allergen extract. On the other hand, in a double-blind, placebo-controlled study performed in 30 dust-mite-allergic children, Hirsch et al. (23) did not observe any consistent clinical benefit in actively treated patients receiving a mean cumulative dose of 0.57 mg of Der p 1. In a recent double-blind controlled study, Bousquet et al. (24) treated asthmatic patients sublingually with house-dust mites for 24 months with a mean cumulative dose of 4.2 mg of Der p 1 (twice the cumulative dose used in our study) and showed a significant improvement of rhinitis symptoms, FEV_1, and peak expiratory flow. The most important feature in our study is the role of the reduction in domestic allergen load observed at 12 and 24 months in both groups, an observation which probably indicates that patients took avoidance measures during the study despite our recommendations. This reduction had a marked impact on modulation of symptom/medication scores and introduced a possible bias in the evaluation of efficacy. This explanation is supported by the reduction of mite-specific IgE observed in the placebo group, probably due to changes in allergen exposure. Sensi et al. (25) reported a significant reduction in nasal and serum specific IgE after 3 months of avoidance of mite allergen.

Concerning safety, no life-threatening adverse event was observed in this study, and the adverse effect rate was very low compared to the data reported in other SLIT studies using allergens of house-dust mite (16–18) or pollen (11–16).

In conclusion, this study showed that SLIT with house-dust-mite extract in perennial allergic rhinitis was well tolerated. In terms of efficacy, the lack of significant clinical improvement in the active group compared to the placebo group was probably due to the effect of the reduction of allergen load due to avoidance measures which interfered with precise evaluation of the effect of SLIT.

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