

Single-allergen Sublingual Immunotherapy *versus* Multi-allergen Subcutaneous Immunotherapy for Children with Allergic Rhinitis

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Summary: It has always been controversial whether a single allergen performs better than multiple allergens in polysensitized patients during the allergen-specific immunotherapy. This study aimed to examine the clinical efficacy of single-allergen sublingual immunotherapy (SLIT) *versus* multi-allergen subcutaneous immunotherapy (SCIT) and to discover the change of the biomarker IL-4 after 1-year immunotherapy in polysensitized children aged 6–13 years with allergic rhinitis (AR) induced by house dust mites (HDMs). The AR polysensitized children ($n=78$) were randomly divided into two groups: SLIT group and SCIT group. Patients in the SLIT group sublingually received a single HDM extract and those in the SCIT group were subcutaneously given multiple-allergen extracts (HDM in combination with other clinically relevant allergen extracts). Before and 1 year after the allergen-specific immunotherapy (ASIT), the total nasal symptom scores (TNSS), total medication scores (TMS) and IL-4 levels in peripheral blood mononuclear cells (PBMCs) were compared respectively between the two groups. The results showed that the TNSS were greatly improved, and the TMS and IL-4 levels were significantly decreased after 1-year ASIT in both groups (SLIT group: $P<0.001$; SCIT group: $P<0.001$). There were no significant differences in any outcome measures between the two groups (for TNSS: $P>0.05$; for TMS: $P>0.05$; for IL-4 levels: $P>0.05$). It was concluded that the clinical efficacy of single-allergen SLIT is comparable with that of multi-allergen SCIT in 6–13-year-old children with HDM-induced AR.

Key words: single allergen; sublingual immunotherapy; multi-allergen; subcutaneous immunotherapy; children; allergic rhinitis

Allergic rhinitis (AR) is a common immunoglobulin (Ig) E-mediated disease with its prevalence increasing worldwide^[1]. Allergen-specific immunotherapy (ASIT) can induce clinical and immunological tolerance toward the offending allergens and therefore greatly ameliorate AR symptoms^[2]. As a conventional ASIT, subcutaneous immunotherapy (SCIT) has been proven to be effective for AR caused by more than two kinds of allergens^[3]. Recently, sublingual immunotherapy (SLIT) has been reported to have similar clinical efficacy to SCIT. Furthermore, a great number of studies on SLIT involved use of just a single allergen^[4,5]. SLIT was demonstrated to inhibit the allergen-driven T cell responses, which leads to the suppression of allergic inflammation, and reduces the circulating antibody levels, particularly allergen-specific immunoglobulin G₄ (IgG₄)^[6]. Many studies showed that SCIT and SLIT had almost the same effects^[7–9].

Polysensitization is much more prevalent (range, 50% to 80%) than monosensitization for patients with moderate-to-severe allergic disease both in the United States and Europe, which has become a crucial factor when choosing allergen extracts for immunotherapy^[10]. There are few clinical trials of ASIT designed to analyze the differences in the effects of immunotherapy in polysensitized patients treated with single and multiple allergen extracts. The clinically relevant allergens of allergy sufferers are identified based on the distribution of

symptoms over the year or provocation test of specific allergens. Nevertheless, when patients are found to be caused by more than one allergen, it is still not clear whether it is more efficacious to use single or multiple allergens immunotherapy. This study aimed to compare the clinical efficacy of single and multiple allergen extracts immunotherapy for polysensitized children sensitized to house dust mites (HDMs) and other allergens and to examine the changes of IL-4 levels in the two modes of administration.

1 MATERIALS AND METHODS

1 Study Subjects

A prospective randomized study was designed to follow up AR patients treated with SCIT or SLIT from July 2010 to October 2011 in order to evaluate the treatment efficacy. Seventy-eight AR patients were recruited for this study during a 3-month period. A total of 39 patients (group A) were randomly assigned to receive SLIT with a single HDM extract, while the remaining 39 patients (group B) to receive SCIT with multiple-allergen extracts (HDM extract in combination with other clinically relevant allergen extracts). The inclusion criteria were as follows: (1) age 6 to 13 years, (2) mild to moderate AR, diagnosed and classified based on the guidance of “Allergic Rhinitis and Its Impact on Asthma” (ARIA)^[11], (3) at least two or more symptoms of sneezing, runny nose, nasal congestion and nasal itching, (4) not only sensitization to the most clinically relevant al-

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lergen HDM, *Dermatophagoides pteronyssinus* (*Der. p*) and *Dermatophagoides farinae* (*Der. f*), but also to other clinically relevant allergens (at least one of them), including grasses, birch, mycete, ragweed and so on. The skin prick test (SPT) for HDM showed strong positive reaction (at least ++ or above), as confirmed by specific immunoglobulin E (IgE) test (>II level). The inhaled allergen test kit (Shanghai New Hualian Pharmaceutical Co., Ltd., China) could detect 20 types of allergens, including HDM, cockroaches, pollen, fungi and so on. The skin prick agent was provided by New-Hualian Union Pharmaceutical Company, China. UniCAP system for specific IgE detection was produced by Pharmacia Company, Sweden.

All subjects were informed of the purpose of this study, treatment protocol and possible results. Written consent was obtained from every subject (or from their

guardians if the subject was less than 13 years old) prior to their participation in the trial and the study was approved by the Ethics Committee of Tongji Hospital, Wuhan, China.

1.2 Allergen Extracts

The single HDM extract (CHANLLERGEN, *Der. f* drops) for SLIT was manufactured by Wolwopharma Biotechnology Company, China. The biologically standardized extracts were labeled with five different concentrations: *Der. f* drops No. 1 (1 µg/mL), No. 2 (10 µg/mL), No. 3 (100 µg/mL), No. 4 (333 µg/mL) and No. 5 (1000 µg/mL). Treatment started with the *Der. f* drops No. 1, and was maintained with the *Der. f* drops No. 4 in children (4–14 years old). The standardized step-up dosage is shown in table 1. All preparations were taken sublingually, kept under the tongue for 1 to 3 min, and then swallowed.

Table 1 Dosages of initiation therapy

Time/Day	<i>Der. f</i> drops No. 1 (week 1)	<i>Der. f</i> drops No. 2 (week 2)	<i>Der. f</i> drops No. 3 (week 3)
1	1 drop	1 drop	1 drop
2	2 drops	2 drops	2 drops
3	3 drops	3 drops	3 drops
4	4 drops	4 drops	4 drops
5	6 drops	6 drops	6 drops
6	8 drops	8 drops	8 drops
7	10 drops	10 drops	10 drops

Subcutaneous desensitization agents for SCIT were manufactured by New-Hualian Union Pharmaceutical Company, China. The multiple-allergen desensitization injections with different compositions and concentrations were prepared according to the results of SPT, sIgE test and the clinical allergy symptoms. Selection of clinically relevant allergens was prioritized, and the number of antigens chosen for each patient was limited to 4 types. The concentrations of subcutaneous injection in the deltoid muscle of the upper arm usually started from 1:10 000. During the conventional treatment of twice a week, the injection dosage started from 0.1 mL, increased by 0.1 mL every time until 1 mL was reached. The next concentration level was then used and the injection was repeated until three months later, the highest concentration (1:100) was achieved and the maintenance therapy started. During the maintenance therapy, the allergen concentration for injection was 1:100 and each dose was 0.5 mL. At the beginning, the frequency of injections was twice a week (total 10 times), and then gradually injection intervals extended according to the efficacy, such as once a week, once two weeks, once four weeks. Each time when the injection interval was changed, the allergen was injected ten times. And it was injected every four weeks in the long-term maintenance therapy. All subjects were confirmed to be in the maintenance phase of treatment at the end of the observation.

1.3 Symptom and Medication Scoring

During the treatment, patients (or their parents for the children) were required to fill out a diary card for scoring the symptoms and medications by the investigators at every visit. Symptoms were scored as previously described^[12]. Nasal symptoms were scored on a scale of

0 to 3, including sneezing (number/day, 0=none, 1=3–5, 2=6–10, and 3= $\geq$ 11), nasal discharge (times/day, 0=none, 1= $\leq$ 5, 2=5–9, and 3= $\geq$ 10), itching (0=none, 1=intermittent itching, 2=tolerable itching, and 3=intolerable itching), and nasal obstruction (0=none; 1=congestion, but no mouth breathing; 2=severe congestion, with occasional mouth breathing; 3=severe congestion, with mouth breathing during the whole day). The total nasal symptom score (TNSS) was the sum of the scores for four individual symptoms (sneezing, nasal discharge, itching and nasal obstruction). Before the treatment, the TNSS of patients in the two groups was evaluated and the peripheral blood samples were collected. The patients were also allowed to use anti-histamine agents and topical glucocorticoid, whenever required. Each oral anti-histamine agent and spray of nasal glucocorticoid was scored as 1^[13]. Patients were followed up on a monthly basis and the total medication score (TMS) obtained. The statistics was performed every 2 months, and the observation period was 1 year.

1.4 Assessment of IL-4 Levels

IL-4 levels were determined by double antibody sandwich ELISA (DAS-ELISA) and the ELISA kit was from DACLONE Company, France. Briefly, the venous blood (5 mL) was harvested into a test tube containing anticoagulant (heparin). Peripheral blood mononuclear cells (PBMCs) were separated and obtained with conventional method, adjusted to 2×10^8 /mL, cultured for 48 h, allowed to adhere for 2 h, washed with normal saline and centrifuged at 2000 r/min for 10 min. At last, the supernatant was collected and stored at -75°C for later use. The levels of IL-4 were detected before and after the treatment. The detection was performed in strict accor-

dance with the kit instruction.

1.5 Statistical Analysis

Statistical analysis was carried out using SPSS 20.0. All tests were two-tailed, and the level of significance was set at 0.05. Two-group *t* test or chi-square test was used for a baseline assessment. *T* test or the Wilcoxon test was employed to determine the difference between groups. Two relevant samples in the group were compared with paired *t* test. All data were shown as $\bar{x} \pm s$.

2 RESULTS

During the study period, 78 patients were eligible for assessment, and a total of 68 patients met the inclusion criteria. The Consolidated Standards of Reporting Trials (CONSORT) flow during screening, recruitment, and randomization is depicted in fig. 1. Table 2 shows the demographic and baseline characteristics of patients.

Table 2 Demographic and clinical characteristics of the study patients at the baseline

Characteristics	Group A (n=34)	Group B (n=34)	P value
Sex (male/female)	16/18	17/17	0.808
Age (years)	9.6±2.5	9.4±2.4	0.862
Antigen numbers	3.0±1.3	2.9±1.1	0.300
Antigen types	6	6	—
TNSS	9.4±1.3	9.8±0.7	0.279
TMS*	115.6±11.6	115.7±11.7	0.959
IL-4 (ng/L)	57.8±7.4	57.8±6.0	0.971

*The statistical analysis of TMS was performed every 2 months.

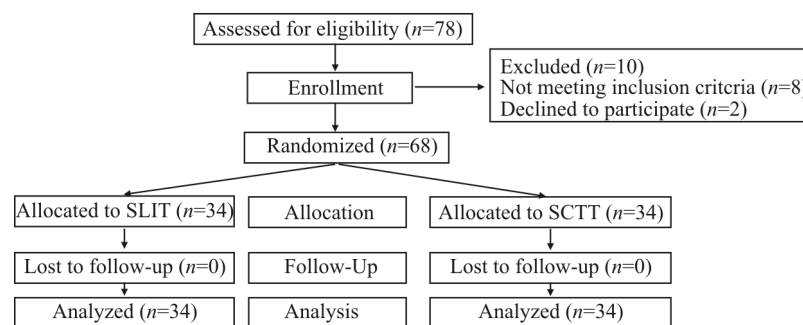


Fig. 1 The Consolidated Standards of Reporting Trials (CONSORT) flow for screening, recruitment, and randomization

2.1 Improvement of AR Symptoms

The total and mean symptom scores of sneezing, nasal discharge, itching, and nasal obstruction before and after 1-year treatment are shown in fig. 2. The TNSS was decreased by 76% (9.4 ± 1.3 vs. 2.3 ± 2.2) in the group A and 77% (9.8 ± 0.7 vs. 2.3 ± 2.4) in the group B, and the differences were significant both in the group A and group B between before and after the treatment ($P < 0.001$). However, there were no significant differences in the TNSS between the two groups after 1-year treatment ($P > 0.05$) (fig. 2).

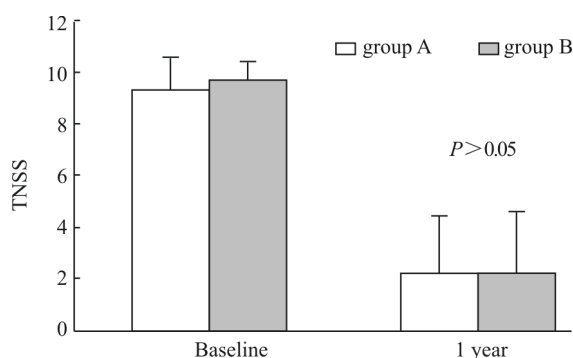


Fig. 2 Comparison of TNSS between groups A and B before and 1 year after the treatment

2.2 Medication Scores

At the end of treatment, the TMS was decreased by 74% (115.6 ± 11.6 vs. 30.4 ± 6.9) in the group A and 73% (115.7 ± 11.7 vs. 30.8 ± 7.1) in the group B, and the differences were significant both in the group A and group B ($P < 0.001$). However, there was also no significant difference in TMS between the two groups after 1-year treatment ($P > 0.05$) (fig. 3).

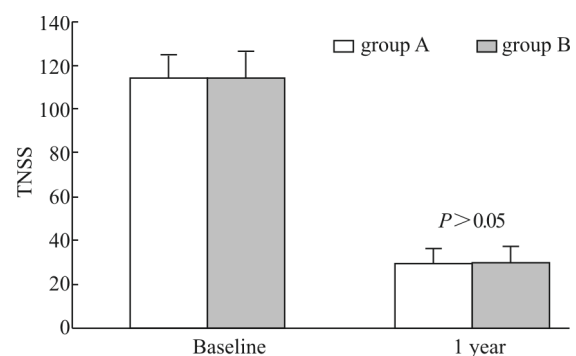


Fig. 3 Comparison of TMS between groups A and B before and 1 year after the treatment

2.3 IL-4 Levels

After 1-year treatment, the levels of IL-4 were decreased by 39% (57.8 ± 7.4 vs. 35.0 ± 4.6) in the group A

and 39% (57.8 ± 6.0 vs. 35.5 ± 4.2) in the group B, and the differences were significant both in the group A and group B ($P < 0.001$). However, also no significant difference was noted in IL-4 levels between the two groups after 1-year treatment ($P > 0.05$) (fig. 4).

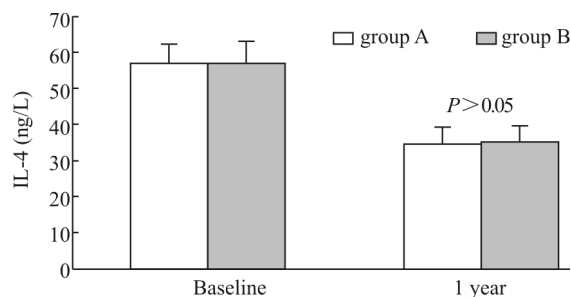


Fig. 4 Comparison of IL-4 levels (ng/L) between groups A and B before and 1 year after the treatment

3 DISCUSSION

In the US, allergy practitioners are accustomed to mix all clinically relevant allergens (mostly in SCIT) for the treatment of polysensitized patients^[14,15]. But, some practitioners believe that the interactions between the allergens make the function of vaccines decline, and a variety of allergens mixing would inevitably make various vaccines diluted^[16]. In Europe, the immunotherapy with the most clinically relevant allergens (mostly in SLIT) is preferred. The single allergen extract is demonstrated to be not only effective for the treatment of monosensitized patients, but also the polysensitized patients^[15]. In addition, few studies have specifically addressed the efficacy of immunotherapy with single vs. multiple allergens. In the research by Amar *et al.*^[17], timothy grass SLIT as a monotherapy was performed to compare with timothy grass SLIT combined with 9 other allergen extracts and with placebo. There were no significant differences in any primary outcome measures (symptom and medication scores, nasal challenge scores, tSPT results, and timothy sIgG₄ levels) between the timothy monotherapy and the multiallergen groups, while there were significant differences in medication or symptom scores between treatment groups and placebo group. Because of a very low grass pollen season in the study year (2008) and the small population size, the results were thought to be questionable by some other scholars.

It is now recognized that allergic disease will be induced when the Th1/Th2 is out of balance^[18]. IL-4 is the key cytokine secreted by Th2 cells and used by many researchers to reflect the balance of Th1/Th2. On one hand, it can promote the development and differentiation of Th2 cells and make Th0 cells differentiate to Th2 cells. On the other hand, it inhibits the function of Th1 cells and blocks the formation of cytokines secreted by Th1 cells, so that the imbalance of Th1/Th2 is aggravated. Furthermore, IL-4 can also induce degranulation of mast cells directly, and the symptoms of allergic disease are aggravated. Currently, the mechanism of ASIT in the treatment of allergic diseases is thought to correct the imbalance of Th1/Th2. Narožna *et al.* concluded that immunotherapy may achieve the therapeutic effect through down-regulating the expression of IL-4^[19]. Cip-

randi *et al.* also found that serum IL-4 appeared to be an early biomarker of immunological response to SLIT^[20]. So the level of IL-4 was applied in our study to reflect the efficacy of single vs. multiple allergen extracts immunotherapy in polysensitized AR patients. Our results showed that the IL-4 levels were significantly decreased after 1-year SLIT and SCIT and there was no significant difference in the change of IL-4 levels between the two groups. SLIT using a single HDM extract almost is not significantly different from SCIT using multiple-allergen extracts (HDM extract plus other clinically relevant allergen extracts) in terms of clinical effects (TNSS, TMS, IL-4) for AR patients sensitized to the most clinically relevant allergen including HDM (*Der. p* and *Der. f*) and other clinically relevant allergens (at least one of them), as grasses, birch, mycete, ragweed and so on. We speculated that the single HDM extract could not only promote the body to produce immune tolerance to the most clinically relevant allergen HDM, but also control other allergens via bystander stimulation^[21].

In conclusion, our study demonstrated that the clinical efficacy of single-allergen SLIT was comparable with that of multi-allergen SCIT in 6–13-year-old children with HDM-induced AR. The effect of immunotherapy with the most clinically relevant allergen HDM may also be extended to allergic diseases caused by other allergens. Although our conclusion provides significant clinical implications, it needs more rigorous confirmations. First, studies should be repeated with the same type of treatment modality and larger samples of patients. Second, more immunological cytokines (e.g. sIgG₄, IL-10) should be used to help elucidate the clinical findings. Finally, more studies need to be designed to yield a consistent conclusion by comparing multiple-allergen SCIT and single-allergen SLIT (e.g. multiple-allergen SCIT vs. single-allergen SCIT, and single-allergen SLIT vs. single-allergen SCIT).

Conflict of Interest Statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

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(Received April 22, 2016; revised Mar. 14, 2016)