

Pharmacology and therapeutics

Clinical efficacy and compliance of sublingual immunotherapy with *Dermatophagoides farinae* drops in patients with atopic dermatitisYu-E Qin¹, MMED, Jing-Ran Mao², MMED, Yue-chan Sang³, MSc, and Wen-Xiu Li³, MSc¹International Health Care Center,²Department of Dermatology, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hang Zhou, and³Department of Clinical Sciences, Zhejiang Wolwo Bio-Pharmaceutical Co., Ltd., Shanghai, China**Correspondence**

Yu-E Qin, MMED

The International Health Care Center

First Affiliated Hospital

College of Medicine

Zhejiang University

Hang Zhou 310003

China

E-mail: qyeyoyo@126.com

Conflicts of interest: None.

doi: 10.1111/ijd.12302

Abstract**Background** The oral route for administration of allergen immunotherapy has been explored since the 1900s. We attempted to evaluate the efficacy and safety of sublingual immunotherapy (SLIT) with *Dermatophagoides farinae* drops in patients with atopic dermatitis (AD) and investigate related factors influencing the patients' compliance.**Methods** A total of 107 patients with AD were randomized to receive either *D. farinae* drops plus pharmacotherapy (treatment group, $n = 58$) or only pharmacotherapy (control group, $n = 49$). Patients' compliance, the total effective rate, daily drug scores, visual analogue scale (VAS) scores, and IgG4 level were compared respectively between two groups at different time points.**Results** Twenty-three cases have withdrawn from the study. The total effective rate in the treatment group (77.78%) was significantly higher than the control group (53.85%) ($P < 0.05$). The treatment group was significantly reduced in daily drug scores and VAS scores compared with the control group at 12 months follow-up. Meanwhile, at the end of therapy, a significant difference was found in the change in average daily drug scores (difference from 1 month) between two groups ($P < 0.01$); The treatment group evidently had a higher level of serum-specific IgG4 than the control group at 6 and 12 month of treatment ($P < 0.05$).**Conclusion** *Dermatophagoides farinae* drops are a safe and effective SLIT for patients with AD, which was proven to reduce the need for medicine. In addition, SLIT could induce a tolerogenic IgG4 response to mite allergen correlated with favorable clinical efficacy. Standardization of specific immunotherapy is essential to ensure therapeutic efficacy and compliance.**Introduction**

Atopic dermatitis (AD) is a chronic, inflammatory skin disease linked to atopic diathesis with a genetic background and is often accompanied by allergic rhinitis or asthma. Dust mite allergen is a common trigger in various risk factors for patients with AD. Allergen-specific immunotherapy (SIT) primarily involved sublingual immunotherapy (SLIT) and subcutaneous immunotherapy (SCIT) and is the only causal treatment currently available that may alleviate the symptoms and affect the natural course of allergic disease. Its beneficial clinical effects have been clearly demonstrated in asthma, rhinitis, and hymenoptera allergy as to both symptoms and medication scores.^{1,2} SLIT has been believed to be painless, easier, and safer than traditional injection SIT.³ Furthermore, It is very prevalent in Europe, Asia, the Middle East, Southern Africa, and Australia. SLIT with standardized extracts of *Dermatophagoides farinae* (*D. farinae* drops, Zhejiang Wolwo

Bio-Pharmaceutical Co., Ltd., De Qing, China) is applied currently in Chinese clinical practice and is performed in a short time (since 2006). Nevertheless, therapeutic compliance plays an important role in the clinical and immunologic effects of SIT. The aim of this study is to evaluate the efficacy and safety of SLIT with *D. farinae* drops in patients with AD. Meanwhile, relevant factors influencing patients' compliance have been observed.

Materials and methods**Study design**

This is a randomized control study. After routine physical examination, allergic history inquiry, and questionnaire, 107 patients were enrolled from March to June 2011. Patients with AD were randomized to receive either SLIT with *D. farinae* drops plus pharmacotherapy (treatment group) or only pharmacotherapy (control group) for 12 months. During this study, based on clinical signs, the treatment group was administered with regular oral

antihistamines and topical medications, which were allowed alone as pharmacotherapy in the control group.

During the treatment, the follow-up was performed at 1, 3, 6, 9, and 12 months of treatment. Simultaneously, patients were called at least every other week, and compliance was observed. Serum specific IgG4 to *D. farinae* (slgG4) was assessed at 1, 6, and 12 months after the beginning of treatment. Therapeutic compliance, effective rate, visual analog scale (VAS) score, and serum IgG4 level were compared between two groups. The use of drugs and extent and frequency of adverse reactions were documented daily using patients' diary card.

The local ethical committee's approval and written informed consent for participation were obtained in this study.

Patients

One hundred and seven patients with AD between 18 and 46 years of age (mean age 27.3 ± 8.2 years) with or without asthma/rhinitis were divided into the treatment group ($n = 58$, 33 men and 25 women) with a mean age of 28.8 ± 7.4 years and the control group ($n = 49$, 30 men and 19 women) with a mean age of 29.8 ± 3.7 years. Inclusion criteria were as follows: (i) clinical history of chronic AD over two years; (ii) moderate AD, diagnosed according to Hanifin and Rajka criteria;⁴ and (iii) sensitization to *D. farinae*, assessed by positive skin prick test (skin wheal area $\geq 50\%$ of the positive control). Exclusion criteria were as follows: (i) any active, acute, or chronic obstructive pulmonary disease, except for asthma and allergic rhinitis; (ii) forced expiratory volume in 1 s $\leq 70\%$ of predicted value; (iii) patients who have disorders with respect to drug absorption, distribution, metabolism, and excretion; and (iv) all contraindications for SLIT or the researchers do not think who would be suitable for study. There were no statistical differences in age, sex, or vital signs (body temperature, respiration, heart rate, and blood pressure) between two groups before therapy ($P > 0.05$).

Skin prick test

Skin prick tests were performed using the Skin Prick Test Kit (Zhejiang Wolwo Bio-Pharmaceutical Co., Ltd.), which included extracts from the spent culture medium of *D. farinae* and the positive (histamine phosphate and glycerol saline solution) and negative control liquid (glycerol saline solution). Twenty minutes after the skin prick test, the skin wheal area was measured. The positive skin prick test was determined when skin wheal areas produced by extracts from the spent culture medium of *D. farinae* were over 50% of skin wheal areas produced by the positive control liquid.

Treatments

In the control group, treatment with only oral levocetirizine hydrochloride tablets and topical Elocon cream (mometasone furoate cream) are permitted.

The treatment group received SLIT with extracts of *D. farinae* and included five treatment dosages with different concentrations (*D. farinae* drops, No.1-5; Zhejiang Wolwo Bio-Pharmaceutical Co., Ltd.). In the first four weeks of SLIT, patients were administrated with increasing doses starting from *D. farinae* drops no. 1 to no. 4, i.e., *D. farinae* drops no. 1 (*D. farinae* protein concentration 1 $\mu\text{g/ml}$) was used in the first week, *D. farinae* drops no. 2 (*D. farinae* protein concentration 10 $\mu\text{g/ml}$) in the second week, and *D. farinae* drops no. 3 (*D. farinae* protein concentration 100 $\mu\text{g/ml}$) in the third week, of those were given respectively as 1, 2, 3, 4, 6, 8, and 10 drops (each drop 40 μl) day after day in a week, *D. farinae* drops no. 4 (*D. farinae* protein concentration 333 $\mu\text{g/ml}$) in the fourth and the fifth week was taken three drops a day. Maintenance therapy with two drops of *D. farinae* drop no. 5 (*D. farinae* protein concentration 1000 $\mu\text{g/ml}$) per day from the sixth week to the 12th month. Patients were instructed to keep the *D. farinae* drops under the tongue for 1–3 minutes and then swallowed. The first dose of *D. farinae* drops was administrated in the physician's office with specific instructions, and after 30 minutes observation, patients could leave the hospital. Regular oral antihistamines and topical medications as the control group were added depending on individual diseases.

During therapy, patients with bacterial infection were allowed to receive therapy with topical Bactroban (mupirocin ointment). A 6-day course of clarithromycin was administrated on any occurrences of skin superinfection. Symptomatic medicine was scored one point respectively for levocetirizine hydrochloride tablet, mometasone furoate cream, and mupirocin ointment use each day and six points for every 6-day course of clarithromycin. The patients were required to record drug use in the diary card.

Outcome measures

Patients' compliance

Patients' reasons and rate of withdrawal were analyzed, and participants failing to complete treatment for 12 months were excluded from the study. The higher the withdrawal rate is, the lower the compliance.

The effective rate evaluation

The severity of AD was evaluated according to the Severity Scoring of Atopic Dermatitis (SCORAD) index,⁵ and therapeutic efficacy was then estimated by the change in SCORAD index. The reduction ratio of the SCORAD index was calculated by the score decrease from the 1st month divided by the 1st month. Patients with a reduction ratio of the SCORAD index $\geq 90\%$ were considered cured, between 60 and 89% were deemed to gain a marked effect, between 20 and 59% were seen to gain improvement, but $\leq 19\%$ were believed to be ineffective. The total effective rate of treatment was expressed as the ratio of cured cases plus cases with marked effect to total cases in each group.

Table 1 Withdrawal cases and reasons for withdrawal in both groups

Reasons	Treatment group (n = 58)			Control group (n = 49)		
	≤6 months	6–12 months	Total	≤6 months	6–12 months	Total
Dissatisfied efficacy	0	0	0	3	2	5
Adverse effects	1	1	2	2	0	2
Cost	3	1	4	0	0	0
Failed to adhere	4	1	5	1	0	1
Symptoms improvement	2	0	2	1	0	1
Lost to follow-up	0	0	0	0	0	0
The others ^a	0	0	0	1	0	1
Total cases	10	3	13	8	2	10

^aThe others included emigrating, suffering from other diseases and so on.

Visual analogue scale

The VAS score based on neurobiophysics and physiology was used to assess the patients' subjective symptoms.⁶ This system links the patient's feelings to an objective representation. As a tool for subjective evaluation, it reflects the quality of life of patients. The efficacy of SLIT was evaluated by an evident improvement of a 10-point VAS. Patients were asked to assess subjectively overall AD symptoms on a 10 cm VAS, where 0 represented no symptoms and 10 indicated maximal symptoms that were cited as extremely severe or discomfort. At each visit, patients were asked to mark a line on the VAS. The physician would then read and record the actual point on the reverse side of the VAS.

Serum sIgG4 assay

Serum *D. farinae*-specific IgG4 was measured by commercial kit (The Binding Site Group Ltd, Birmingham, UK). IgG4 was expressed in mg/L.

Statistical analysis

Statistical analysis was performed using SPSS16.0 software (SPASS, Inc., Chicago, IL, USA); continuous variables were expressed as mean ± SD. Incidence was presented as number or percentage. Statistical differences between means of two groups were tested using the *t*-test and chi-squared test. $P < 0.05$ were considered significant.

Results

Patient compliance

As shown in Table 1, 23 cases have withdrawn from the study. Thirteen cases in the treatment group yielded a withdrawal rate of 22.4%, and 10 cases in the control group produced a withdrawal rate of 20.4%; however, there was no difference in withdrawal rate between the two groups ($\chi^2 = 0.1189$, $P > 0.05$) at the end of treatment as well as in the first six months of treatment ($\chi^2 = 0.2842$, $P > 0.05$), in which the highest withdrawal rates were observed—withdrawal patients accounted for 76.9% of the total withdrawal population in the treatment group, 80.0% in the control group. The first two reasons for withdrawal in the treatment group were that patients failed to adhere to therapy and could not bear the cost, but in the control group, they were dissatisfied efficacy and adverse effects.

Clinical efficacy

The treatment group had a total efficacy rate of 77.78% (35 of 45), which was significantly higher than 53.85% (21 of 39) in the control group ($\chi^2 = 12.73$, $P < 0.05$) (Table 2).

Drug scores

When average daily drug scores were compared at different time points, there was no difference between the two groups ($P > 0.05$) at one month follow-up; whereas, the treatment group was significantly lower compared with the control group at both 6 and 12 months follow-up ($t_{6 \text{ months}} = 5.304$, $t_{12 \text{ months}} = 6.536$, $P < 0.01$). Moreover, at 12-month follow-up compared with the first

Table 2 Efficacy of treatment in two groups

Group	n	Cure n (%)	Marked effect n (%)	Improvement n (%)	ineffective n (%)	Total efficacy rate
Treatment group	45	11 (24.4%)	24 (53.3%)	9 (20.0%)	1 (2.2%)	77.78%*
Control group	39	6 (15.4%)	15 (38.5%)	13 (33.3%)	5 (12.8%)	53.85%
Total	84	17 (20.2%)	39 (46.5%)	22 (26.2%)	6 (7.1%)	

Total efficacy rate was expressed as the ratio of cured cases plus cases with marked effect to total cases in each group. Compared with the control group, * $P < 0.05$.

Table 3 Daily drug scores at different time points

Group	n	Average daily drug scores		
		1 month	6 month	12 month
Treatment group	45	2.1 ± 0.5	1.2 ± 0.6**	0.5 ± 0.4**
Control group	39	2.2 ± 0.6	1.8 ± 0.4	1.3 ± 0.7

Compared with the control group, ** $P < 0.01$.

month, the decrease of average daily drug scores in the treatment group were significantly greater than that in the control group ($t = 5.76$, $P < 0.01$) (Table 3).

Visual analog scores and serum sIgG4

At the end of treatment, the VAS scores declined significantly in the treatment group in contrast with the control group ($t = 2.426$, $P < 0.05$). In addition, the treatment group had an evidently higher level of serum sIgG4 than the control group at both 6 and 12 months of treatment ($t_{6 \text{ months}} = 6.081$, $t_{12 \text{ months}} = 12.828$, $P < 0.01$) (Table 4).

Safety evaluation

There were no severe adverse effects observed during therapy. Even though three patients with transient oral itching and one patient with gastrointestinal discomfort in the treatment group were found, three cases had skin flushing in the control group. However, the above adverse events spontaneously disappeared without therapy. Besides, 11 patients (six in the treatment group and five in the control group) underwent repeated exacerbations of allergic symptoms, and their symptoms alleviated after dose adjustment and using symptomatic medication. No statistical difference was observed with respect to incidence of adverse reaction between the treatment group and the control group ($\chi^2 = 0.0488$, $P > 0.05$).

Discussion

This 12-month randomized control study has confirmed the efficacy and safety of SLIT with *D. farinae* drops in

the treatment of patients with AD. SLIT has been recommended recently by the World Allergy Organization as a new therapeutic option for patients with allergic rhinitis, asthma, and AD,⁷ representing an alternative to subcutaneous immunotherapy.² Studies have confirmed the efficacy of SLIT in reducing SCORAD index and drug consumption for patients with AD. A study reported by Cadario *et al.*⁸ in patients with AD ($n = 86$, aged 3–60 years) showed that 51 subjects had a significant improvement defined by a SCORAD reduction of >30% after one year of SLIT with house dust mite extracts. Meanwhile, a trend in reducing the usage of topical corticosteroid and calcineurin inhibitors was also observed. In our study, after 12 months of SLIT treatment, 35 of 45 patients had a SCORAD reduction of ≥60% with significantly low drug scores. These findings are also supported by the apparent improvement in VAS, which was in favor of the study by Ma and Duo.⁹ The double-blind, placebo-controlled study by Silny and Czarnecka-Operacz¹⁰ suggested that two of 10 patients with AD had severe exacerbation under SCIT, which resulted in 20% of inefficient rate, as compared to 2.2% (1 of 45) in our study.

It is estimated that 70–80% of patients with AD are associated with IgE-mediated allergic sensitization and eosinophilia, the elevated IgE responses and eosinophilia are mainly caused by an increased expression of T-helper (Th) 2 cytokines, which include the production of interleukin (IL)-4, IL-13, and IL-5.^{11,12} In addition, in chronic AD, IL-12 production by the infiltrated eosinophils and macrophages can induce the switch to Th1 responses.¹³ In SLIT, allergen is captured by Langerhans-like dendritic cells (DCs) in the oral mucosa, and DCs will then mature and migrate to proximal draining lymph nodes. Within lymph nodes, DCs present allergen to T cells in the context of protolerogenic signals, causing the induction of regulatory T cells that inhibit effector T cells (e.g., Th1, Th2) through secreting the suppressive cytokines IL-10 and transforming growth factor- β and/or by cell–cell contact; IL-10 and transforming growth factor- β enhance IgG4 and IgA production in B cells, respectively.^{14,15} Although the relationship between IgG4 and efficacy of allergen-specific immunotherapy is still controversial,

Table 4 VAS at the end of treatment and serum IgG4 level at different time points

Group	n	VAS At the end of treatment	sIgG4 (mg/L)		
			1 month	6 months	12 months
Treatment group	45	6.10 ± 2.16*	372.9 ± 121.8	528.1 ± 141.5**	687.5 ± 150.3**
Control group	39	7.20 ± 2.67	397.4 ± 116.8	388.4 ± 107.6	369.7 ± 112.7

VAS, visual analog scale.

Compared with the control group, * $P < 0.05$, ** $P < 0.01$.

several investigators have stated that IgG subclasses, mainly IgG4, have a protective role, which is attributed to suppress basophil and mastocyte degranulation as well as IgE-facilitated antigen presentation by competing with IgE for binding to allergen.¹⁶ Thereby this protective activity may make the effectiveness for immunotherapy. As shown in a meta-analysis of six SLIT studies on serum antibody level,¹⁷ our data again confirm a consistent increase in serum allergen-specific IgG4 after SLIT. Furthermore, after 70 days of house dust mite extract-SIT, a significant increase in specific IgA, IgG1, and IgG4 was observed though no change in specific IgE.¹⁸ Some SLIT studies observed a decrease in the IgE/IgG4 ratio.^{19–21} These findings suggested that SLIT start to work and to regulate allergen-specific antibody.

Patient compliance plays a key role in successfulness of SLIT, but there is far little attention being paid to this aspect. In SLIT, patients are administered at home by themselves. Although SLIT reduces the pain of injections from SCIT and inconvenience from frequent hospital admissions, non-standardization and discontinuing of therapy probably happen with lack of physician guidance and supervision.

A survey conducted by Passalacqua *et al.*²² was a real-life study investigating SLIT compliance in adult and adolescent patients and found a withdrawal rate of only 6.7% during the six months of observation. The major reasons for dropping out were: unknown; followed by side effects (not related or possibly related to SLIT); did not begin the treatment; required to be switched to injection immunotherapy; unaffordable cost; and the other unexpected events (intercurrent surgery, car accident, pregnancy).²² The study by Marogna *et al.*²³ suggested that 15% of patients dropped out in the SLIT group ($n = 319$) during the three years of the controlled study. By comparison, our study showed a low compliance (22.4% of withdrawal rate). In a 1-year SLIT with *D. farinae* drops, Chen *et al.*²⁴ demonstrated that the dropout rate was 45.19% in the general treatment group with the normal clinical procedure but only 18.25% in the intervention treatment group provided with systemic patient management, including patient education, common problems of SLIT in real life, regular telephone interviews, and feedback. In this study, there were three major reasons for dropouts, such as no improvement of symptoms, no further consultation because of too far away or too busy, and side effects; more than half of dropouts happened in the first two months. We found a high withdrawal rate during six months of treatment. The use of standardized SLIT was approved by the Chinese State Food and Drug Administration in 2006. Since then a large number of outpatients were recommended for SLIT. Among them, many patients did not receive full

instructions or proper training by attending physicians before starting SLIT. Therefore, there was a high dropout rate occurring in the early stages of SLIT. In the interests of ideal patient compliance, sociological, economic, and familial factors should be taken into consideration when describing SLIT.²⁵ Meanwhile, patient education and management should be introduced because it represents a critical factor in enhancing compliance for SLIT.²⁶

Commonly reported adverse effects were not observed during the period of SLIT in our study. It is reported that adverse effects may give rise to low compliance rate.²⁷ During SLIT, side effects generally occur in the building-up phase, in which symptomatic medication can be used together. There is a need for patients to know potential adverse reactions before treatment, and then patients can psychologically prepare and inform doctors on side effects when they appear so that they can be dealt with in a timely manner.

In conclusion, our study confirmed that SLIT coupled with a regular anti-allergic drug is effective, safe, and convenient. Patient education should be brought into the overall course of SLIT, especially in the early stage of treatment (within six months). It is not only helpful for patients to have a correct understanding of their disease but also the indications and implications of SLIT, which will improve patient compliance and efficacy, and then long-term efficacy can be achieved.

References

- 1 Watanabe AS, Fonseca LAM, Galvão CES, *et al.* Specific immunotherapy using *Hymenoptera* venom: systematic review. *Sao Paulo Med J* 2010; **128**: 30–37.
- 2 Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998; **102**: 558–562.
- 3 Della Volpe A, D'Agostino GW, Varricchio AM, *et al.* Sublingual allergen-specific immunotherapy in allergic rhinitis and related pathologies: efficacy in a paediatric population. *Int J Immunopathol Pharmacol* 2002; **15**: 35–40.
- 4 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; **92** (Suppl.): 44–47.
- 5 Stalder JF, Taieb A, Atherton DJ. Severity scoring of atopic dermatitis: the SCORAD Index. *Dermatology* 1993; **186**: 23–31.
- 6 Pajno GB, Caminiti L, Vita D, *et al.* Sublingual immunotherapy in mite-sensitized children with atopic dermatitis: a randomized, double-blind, placebo-controlled study. *J Allergy Clin Immunol* 2007; **120**: 164–170.
- 7 Canonica GW, Bousquet J, Casale T, *et al.* Sub-lingual immunotherapy: World Allergy Organization position paper 2009. *Allergy* 2009; **64**(Suppl.): 1–59.

- 8 Cadario G, Galluccio AG, Pezza M, *et al.* Sublingual immunotherapy efficacy in patients with atopic dermatitis and house dust mites sensitivity: a prospective pilot study. *Curr Med Res Opin* 2007; 23: 2503–2506.
- 9 Ma XP, Duo LK. Efficacy of sublingual immunotherapy in children with dust mite allergic asthma. *Chin J Contemp Pediatr* 2010; 12: 344–347 (in Chinese).
- 10 Silny W, Czarnecka-Operacz M. Specific immunotherapy in the treatment of patients with atopic dermatitis—results of double blind placebo controlled study. *Pol Merkur Lekarski* 2006; 21: 558–565.
- 11 Novak N, Bieber T. Allergic and nonallergic forms of atopic diseases. *J Allergy Clin Immunol* 2003; 112: 252–262.
- 12 Leung DYM. Atopic dermatitis: the skin as a window into the pathogenesis of chronic diseases. *J Allergy Clin Immunol* 1995; 96: 302–319.
- 13 Leung DYM. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000; 105: 860–876.
- 14 Moingeon P, Batard T, Fadel R, *et al.* Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* 2006; 61: 151–165.
- 15 Scadding G, Durham S. Mechanisms of sublingual immunotherapy. *J Asthma* 2009; 46: 322–334.
- 16 Fujita H, Soyka MB, Akdis M, *et al.* Mechanisms of allergen-specific immunotherapy. *Clin Transl Allergy* 2012; 2: 1–8.
- 17 Wilson DR, Torres Lima M, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2004; 60: 4–12.
- 18 Jutel M, Akdis M, Budak F, *et al.* IL-10 and TGF- β cooperate in regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; 33: 1205–1214.
- 19 La Rosa M, Ranno C, André C, *et al.* Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 1999; 104: 425–432.
- 20 Troise C, Voltoni S, Canessa A, *et al.* Sublingual immunotherapy in *Parietaria* pollen-induced rhinitis: a double-blind study. *J Invest Allergol Clin Immunol* 1995; 5: 25–30.
- 21 André C, Perrin-Fayolle M, Grosclaude M, *et al.* A double-blind placebo-controlled evaluation of sublingual immunotherapy with a standardized ragweed extract in patients with seasonal rhinitis. *Int Arch Allergy Immunol* 2003; 131: 111–118.
- 22 Passalacqua G, Musarra A, Pecora S, *et al.* Quantitative assessment of the compliance with a once-daily sublingual immunotherapy regimen in real life (EASY Project: evaluation of a novel SLIT formulation during a year). *J Allergy Clin Immunol* 2006; 11: 946–948.
- 23 Marogna M, Spadolini I, Massolo A, *et al.* Randomized controlled open study of sublingual immunotherapy for respiratory allergy in real-life: clinical efficacy and more. *Allergy* 2004; 59: 1205–1210.
- 24 Chen JJ, Kong WJ, Xiang JS. Patient compliance with sublingual immunotherapy and its countermeasure. *J Clin Otorhinolaryngol Head Neck Surg* 2010; 24: 203–206 (in Chinese).
- 25 Pham-Thi N, de Blic J, Scheinmann P. Sublingual immunotherapy in the treatment of children. *Allergy* 2006; 61(Suppl. 81): 7–10.
- 26 Incorvaia C, Rapetti A, Scurati S, *et al.* Importance of patients education in favouring compliance with sublingual immunotherapy. *Allergy* 2010; 65: 1341–1342.
- 27 Di Rienzo V, Minelli M, Musarra A, *et al.* Post-marketing survey on the safety of sublingual immunotherapy in children below the age of 5 years. *Clin Exp Allergy* 2005; 35: 560–564.