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Changes in Serum Specific IgG4 and IgG4/IgE Ratio in Mite-Sensitized Taiwanese Children with Allergic Rhinitis Receiving Short-Term Sublingual-Swallow Immunotherapy: A Multicenter, Randomized, Placebo-Controlled Trial

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SUMMARY The aim of this study was to evaluate the clinical and immunologic effects of sublingual-swallow immunotherapy (SLIT). A six-month, multicenter, double-blind, placebo-controlled trial was carried out in 59 patients aged 6 to 18 years with allergic rhinitis who were sensitized to mites only. Patients were randomly assigned to placebo or SLIT with a standardized *Dermatophagoides pteronyssinus (D.p.)/D. farinae (D.f)* 50/50 extract. Nasal symptom scores and use of medications were recorded. Skin sensitivity, mite-specific IgE, IgG4, and IgG4/IgE were evaluated before and after treatment. The skin sensitivity, total nasal symptom scores and medication consumption did not differ significantly after treatment. Specific IgG4 (both $p < 0.001$) and IgG4/IgE to *D.p.* and *D.f* ($p = 0.010$, $p = 0.001$, respectively) increased significantly in the treatment group. Specific IgE increased significantly in both placebo and SLIT groups after treatment but did not differ between the two groups. The medication was well tolerated. SLIT did not significantly improve clinical manifestations of allergic rhinitis when used for 6 months. We demonstrated SLIT did significantly increase specific IgG4 and IgG4/IgE compared to treatment with placebo.

The prevalence of allergic rhinitis is rapidly increasing in many countries.[1,2] Although it is not life-threatening, this disorder is important because of its impact on the quality of life. Among a number of therapeutic options for allergic rhinitis is allergen-specific immunotherapy.

Subcutaneous immunotherapy is clinically effective for allergic disorders like rhinoconjunctivitis or asthma.[3,4] However, the need for injections is inconvenient, and the treatment is occasionally associated with severe local or systemic side effects or even death.[4,5] Sublingual immunotherapy (SLIT) has been demonstrated to be a safe and effective alternative to parenteral injection therapy.[6] This form of treatment is particularly attractive in the pediatric population as it is more likely than injections to be well tolerated.

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House dust mites are the most important allergen in Taiwan,[1] but the results of trials of SLIT to desensitize children to this allergen have been mixed. While SLIT appears to be safe, only one of three double-blind placebo-controlled trials in mite-sensitized children with allergic rhinitis demonstrated symptom improvement.[8-10] Besides, some reports suggested that induction of IgG antibodies may be important for successful allergen immunotherapy. We designed this study using a high dose of mite allergens for a short treatment period to evaluate the clinical and immunologic effects of SLIT in Taiwanese children with allergic rhinitis who are sensitized by mites.

MATERIALS AND METHODS

Patients

Children aged 6 to 18 years who were followed in the pediatric clinics of 3 medical centers in Taiwan and who had at least a two-year history of allergic rhinitis and were recruited. Informed consent was obtained from all the parents or guardians of all study subjects. The diagnosis of allergic rhinitis was based on history, a positive skin test for house dust mites, and the presence of serum specific IgE (≥ 3 on the MAST CLA allergen test; Hitachi Chemical Diagnostics, Inc., California, USA). Patients were excluded if they were sensitive to cockroaches, *Alternaria, Cladosporium,* dog or cat danders, or pollens by skin prick test (wheal ≥ 5 mm) or specific IgE (≥ 1+ by MAST CLA). Thus, all subjects were sensitive to house dust mites only. Patients who had previously received any form of immunotherapy, had contraindications to specific-allergen immunotherapy, or were being treated with oral or parenteral steroids, cromolyn, long-acting antihistamines, or botanicals or other medicines which would interfere with the evaluation were excluded. We also excluded those with any anatomical abnormality of the upper respiratory tract, peptic ulcer, reflux esophagitis, cardiovascular disease, or severe persistent asthma. Among 89 children with allergic rhinitis who were screened, 63 children were enrolled. They were randomly assigned to receive either SLIT (30 patients) or placebo therapy (33 patients). Four patients (2 in each group) were withdrawn or terminated from the study because of adverse effects, lack of efficacy, loss to follow-up, or withdrawal of consent, leaving 59 patients who completed the study, 28 using SLIT and 31 receiving placebo. Intention-to-treat analysis was carried out. Only those who completed the study were included in the outcome analysis. The human ethical and clinical trial committee in each study center has approved this trial.

Study design

The study was conducted over 26 weeks, including 2 weeks of screening and collection of baseline data, 3 weeks of induction therapy, and 21 weeks of maintenance therapy. Baseline screening included a medical history, including any medications the child was on; a physical examination; and standard laboratory tests, including hematology, biochemistry, and a urinalysis.

Treatment

Staloral (Stallergenes, France), a standardized extract of equal amounts of allergens from the house dust mites, *Dermatophagoides pteronyssinus (D.p.)* and *D. farinae (D.f),* was used for SLIT. The Staloral was formulated in three strengths: 10, 100, and 300 IR/ml. The extract and placebo were dispensed in the same glycerol saline diluents, so that both investigator and subjects were blinded to the solution being used. Vials were labeled indicating which weeks they were to be used. The study solution was taken sublingually in the morning before breakfast, with the drops to be kept in the mouth for at least 2 minutes and then swallowed. During the 3-week induction period, an accelerated dosing schedule was used. For the first week, the SLIT vial contained 10 IR/ml and subjects were instructed to start with one drop and gradually increase to 10 drops by day 7. Beginning on day 8, they switched to the second-week vial, which for the SLIT group contained 100 IR/ml. They were instructed to start with 1 drop per day and gradually increase the dose to 20 drops on day 14. The third-week and subsequent maintenance vials all contained Staloral 300 IR/ml. On day 15, patients were to take 7 drops and increase the dose to 20 drops on day 19. This was the maintenance dose which was then used for another 21 weeks. If taken as directed, the cumulative dose would be 37,312 IR, equivalent to 1.56 mg of *D.p.* and 2.71 mg of *D.f.* Patients in the placebo group followed exactly the same pattern of increasing doses of the placebo glycerol saline diluent.

Diary card

The patients' parents were provided with diary cards on which they recorded nasal symptom scores and medication use throughout the run-in and treatment periods. Nasal symptoms (sneezing, rhinorrhea, nasal itching, and nasal obstruction) were each reported on a four-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe). The total nasal symptom score was the sum of the 4 individual symptom scores. During the trial, patients were allowed to take the following medications if needed: antihistamine (fexofenadine) and inhaled β2-agonist (albuterol sulfate HFA inhalation aerosol). The number of tablets and/or puffs were recorded on the diary cards. Any adverse events were also recorded.

Main outcome measure

The primary outcome assessed in this study was the change in the mean total nasal symptom score from baseline to the end of the study.

Secondary outcome measures

We also assessed changes in skin prick tests and serum specific antibody levels. Before treatment and at the end of the trial, skin prick testing was performed in duplicates on the forearm by using a standardized allergen panel (Stallergenes, France) for *D.p.* and *D.f.,* cockroach, cat and dog dander, *Alternaria, Cladosporium* and pollen mix extracts. A mean wheal diameter of ~5 mm or a wheal with > 50% of the diameter of a positive control wheal was considered a positive result. Before treatment and at the end of the trial, specific IgE and IgG4 to *D.p.* and *D.f.* was determined by Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). The results of specific IgE and IgG4 were expressed in μg/l, and the IgG4/IgE ratio was also calculated.

Statistical methods

Two-way analysis of variance (ANOVA) was used to compare the SLIT and treatment groups by fixed factor treatment, center, and treatment-by-center interaction as terms in the model for continuous variables. The treatment-by-center interaction was considered statistically significant if the *p* value for the interaction was smaller than 0.10. The paired *t* test was used to analyze changes from baseline within each treatment group. Cochran-Mantel-Haenszel statistics for center control for categorical variables were used to compare differences among the SLIT and placebo groups.

RESULTS

Patients

The patients in both groups had similar characteristics, including gender, age, weight, height and allergic rhinitis history (Table 1). None of the subjects smoked or used alcohol.

Table 1 Demographic and clinical characteristics of enrolled population

Characteristics	**Staloral,** n = 30	**Placebo,** n = 33	***p*-value**
Gender			0.762*
Male	22 (73%)	23 (70%)	
Female	8 (27%)	10 (30%)	
Age			0.927+
Mean ± SD (years)	9.7 ± 3.3	9.7 ± 3.0	
Weight (kg)			0.726+
Mean ± SD (kg)	36.7 ± 14.6	38.0 ± 16.3	
Height (cm)			0.796+
Mean ± SD (cm)	138.2 ± 17.3	139.2 ± 16.4	
Allergic rhinitis history			0.520+
2-5 years	19 (63%)	17 (52%)	
6-10 years	10 (33%)	16 (48%)	
13 years	1 (3%)	0 (0%)	

*Cochran -Mantel-Haensze1 statistics with center control
+Treatment effect by two-way ANOVA with treatment, center, and treatment-by-center-interaction

Symptom scores

In children receiving SLIT, there was slight but statistically insignificant improvement in mean total nasal symptom scores (1.79 ± 1.13 at baseline *versus* 1.72 ± 1.78 at week 24, $p = 0.826$). A similar, statistically insignificant improvement was seen in the placebo group (2.33 ± 1.62 at baseline *versus* 1.89 ± 1.90 at week 24, $p = 0.095$). There were no significant differences between the two groups in mean total nasal and individual symptom scores at baseline nor in the changes in those scores from baseline to week 24 (Table 2).

Medication use

During the run-in period and throughout the study, the use of medications did not differ significantly between the two groups (Table 3). The baseline consumption of antihistamine appeared to be higher in the placebo (0.62 ± 0.65 tablet/day) compared to the SLIT (0.38 ± 0.44 tablet/day) group, but the difference did not achieve statistical significance *(p* = 0.055). There was very little use of β2-agonists in either group at baseline. In neither group was there a significant change in the daily amount of medication used through the study.

Skin prick test

At the end of treatment, the skin sensitivity to *D.p.* was unchanged in the SLIT group (5 subjects having a negative test at baseline and at 24 weeks). In the placebo group, 1 had a negative skin test to *D.p.* at baseline, but 4 had negative tests at the end of the study. However, the between-treatment differences in skin reactivity were not statistically significant *(p* = 0.188). In the SLIT, the number of children with

Table 2 Total nasal symptom scores (daily, daytime, and nighttime)

Total nasal symptom scores*	Statistical parameters	Staloral (n = 28)			Placebo (n = 31)			Group difference	
		Baseline	Week 24	Change from baseline	Baseline	Week 24	Change from baseline	Baseline	Change from baseline
Daily#	Mean ± SD.	1.79 ± 1.13	1.72 ± 1.78	-0.07 ± 1.82	2.33 ± 1.62	1.89 ± 1.90	-0.44 ± 1.47		
	p-value	0.826*			0.095*			0.265+	0.608+
Daytime	Mean ± SD	1.96 ± 1.30	1.85 ± 1.86	-0.10 ± 2.06	2.61 ± 1.79	2.07 ± 1.94	-0.54 ± 1.58		
	p-value			0.786*			0.058*	0.098+	0.824+
Nighttime	Mean ± SD	1.63 ± 1.11	1.58 ± 1.73	-0.04 ± 1.66	1.05 ± 1.56	1.71 ± 1.92	-0.34 ± 1.55		
	p-value			0.885*			0.212*	0.169+	0.665+

*0, no symptoms; 1, mild; 2, moderate; 3, severe.
#(Daytime scores + Nighttime scores) + 2.
*Paired *t* test.
+Treatment effect by two-way ANOVA with treatment, center, and treatment-by-center-interaction.

Table 3 Use of medications

Rescue medications	Statistical parameters	Staloral (n = 28)			Placebo (n = 31)			Group difference	
		Baseline	Week 24	Change from baseline	Baseline	Week 24	Change from baseline	Baseline	Change from baseline
Antihistamine (tablets/day)	Mean ± SD	0.38 ± 0.44	0.25 ± 0.51	-0.31 ± 0.52	0.62 ± 0.65	0.53 ± 0.69	-0.09 ± 0.50		
	p-value			0.826*			0.312*	0.055+	0.462+
β_2-agonist (puffs/day)	Mean ± SD	0.04 ± 0.13	0.04 ± 0.12	-0.00 ± 0.15	0.05 ± 0.17	0.04 ± 0.15	-0.01 ± 0.20		
	p-value			0.932*			0.843*	0.836+	0.748+

*Paired *t* test.
+Treatment effect by two-way ANOV A with treatment, center, and treatment-by-center-interaction.

negative skin tests to *D.f.* increased from 1 at baseline to 6 at the end of treatment, while in the placebo group, 6 had negative tests at baseline compared with 5 at 24 weeks. Again, however, the between-treatment differences in *D.f* skin test results did not differ significantly *(p* = 0.926) (Table 4).

Table 4 Skin reactivity to *D. pteronyssinus* and *D. farinae*

	D. pteronyssinus					***D. farinae***				
	Staloral (n = 28)		**Placebo** (n = 31)		***p*-value***	**Staloral** (n = 28)		**Placebo** (n = 31)		***p*- value***
	Negative	**Positive**	**Negative**	**Positive**		**Negative**	**Positive**	**Negative**	**Positive**	
Baseline	5 (18%)	23 (82%)	1 (3%)	30 (97%)	0.050	1 (4%)	27 (96%)	6 (19%)	25 (81%)	0.077
Week 24	5 (18%)	23 (82%)	4 (13%)	27 (87%)	0.188	6 (21%)	22 (79%)	5 (16%)	26 (84%)	0.926

*Cochran-Mantel-Haenszel statistics with center control.

Table 5 Serum specific IgE to *D. pteronyssinus* and *D. farinae*

Specific IgE (μg/l)	**Statistical parameter**	**Staloral group,** n = 28		**Control group,** n = 31		**Group difference**	
		Baseline to 24th week	**Change from baseline**	**Baseline to 24th week**	**Change from baseline**	**Baseline**	**Change from baseline**
D. ptero-nyssinus	**Mean ± SD** (μg/l)	129.7 ± 91.0		98.8 ± 71.5			
			40.8 ± 76.1		21.0 ± 46.7		
		170.5 ± 88.8		119.8 ± 85.5			
	***p*-value**		0.008*		0.018*	0.151+	0.124+
D. farinae	**Mean ± SD** (μg/l)	108.1 ± 79.5		83.3 ± 62.9			
			49.0 ± 73.9		24.2 ± 43.3		
		157.1 ± 91.4		107.5 ± 78.7			
	***p*-value**		0.002*		0.004*	0.173+	0.087+

*Paired *t* test
+Treatment effect by two-way ANOV A with treatment, center, and treatment-by-center-interaction.

Table 6 Serum specific IgG4 to *D. pteronyssinus* and *D. farina*

Specific IgG4 (μg/l)	**Statistical parameter**	**Staloral** (n = 28)		**Placebo** (n = 31)		**Between group difference**	
		Baseline to 24th week	**Change from baseline**	**Baseline to 24th week**	**Change from baseline**	**Baseline**	**Change from baseline**
D. ptero-nyssinus	**Mean ± SD** (μg/l)	591.4 ± 476.9		520.1 ± 308.2			
			772.9 ± 1,002.8		-92.4 ± 290.1		
		1,364.3 ± 1,293.0		427.7 ± 307.7			
	***p*-value**		< 0.001*		0.018*	0.553+	< 0.001+
D. farinae	**Mean ± SD** (μg/l)	425.0 ± 392.1		386.1 ± 285.8			
			710 ± 990.9		-6.4 ± 280.1		
		1,135.1 ± 1,231.1		379.7 ± 306.4			
	***p*-value**		0.002*		0.889*	0.721+	< 0.001+

*Paired *t* test.
+Treatment effect by two-way ANOV A with treatment, center, and treatment-by-center-interaction.

Mite- specific serum IgE and IgG4, IgG4/IgE

Serum specific IgE was significantly higher in both groups after the treatment ended, but the levels did not differ significantly between the two groups (Table 5). The SLIT group, however, had a significantly greater increase in specific IgG4 levels to both *D.p.* and *D.f* and compared with the placebo group (Tables 6, 7). The *D.f.*-specific IgG4/IgE ratio increased significantly in the SLIT group, and both mite-specific IgG4/IgE ratios were significantly greater in the SLIT group at the end of treatment compared with the placebo group.

Adverse events

The medication was well tolerated by all patients, and no severe drug-related adverse events were reported. Twenty six patients (19 in the active group and 7 in the placebo group) had mild adverse events, including tongue numbness, the most common, followed by epistaxis, mouth ulceration, and asthma attacks. One patient discontinued the study because of a moderate asthma attack that was not drug-related.

DISCUSSION

This study compared the efficacy and safety of SLIT using high-dose standardized mite extracts *versus* placebo in children with allergic rhinitis who were sensitized to mites only. We could not demonstrate any significant difference in clinical outcome, either by symptom scores or medication use after 24 weeks treatment.

Tari *et al.*[10] found a significant reduction in rhinitis symptom scores in mite-sensitized children after 12 and 18 months of SLIT. Bahceciler *et al.*[11] demonstrated a significant decrease in mean daily doses of intranasal steroids needed to control rhinitis symptoms after SLIT for 6 months. Two other studies, one for 24 and one for 12 months, found no consistent clinical benefit of SLIT compared to placebo.[8,9] The discrepancies may be due to the differences in duration of treatment, dose and dosing schedule, the way symptoms were evaluated, or the use of allergen avoidance measures. Several factors may explain the absence of statistically significant differences between groups in our study. Firstly, the failure to show any clinical effect was probably due to the insufficient duration of the treatment. The duration of treatment may not have been long enough to achieve clinical relevant results. There are still no experimental data on the optimal duration for SLIT.[12] Secondly, the optimal frequency of maintenance dosing has not yet been identified and dosing recommendations vary from daily to once weekly application. A daily application of high peak dose might be the optimal treatment schedule.[13] The third important factor for the efficacy of SLIT could be the extended contact time with the sublingual mucosa. Pfaar *et al.*[13] sug-

Table 7 Ratio of IgG4/IgE to *D. pteronyssinus* and *D. farinae*

IgG4/ IgE	Statistical Parameter	Staloral group (n = 28)		Control group (n = 31)		Group difference	
		Baseline to 24th week	Change from baseline	Baseline to 24th week	change from baseline	Baseline	Change from baseline
D. pteronyssinus	Mean ± SD	8. 77 ± 11.68		9.23 ± 12.38			
			9.45 ± 31.03		-2.63 ± 4.35	-0.46	12.08
		18.23 ± 41.67		6.59 ± 9.24			
	p-value		0.119*		0.002*	0.918'	0.01+
D. farinae	Mean ± SD	5.20 ± 4.72		8.62 ± 14.76			
			4.49 ± 6.43		-2.00 ± 5.56	-3.42	6.49
		9.69 ± 9.87		6.62 ± 11.35			
	p-value		0.001 *		0.054*	0.218+	0.001+

*Paired *t* test.
+Treatment effect by two-way ANOV A with treatment, center, and treatment-by-center-interaction.

gested to keep the contact time with the sublingual mucosa as long as possible (preferably 3 minutes). Further work for concerning the influence of contact time is needed to evaluate the clinical efficacy and mechanism of action of SLIT.

Although allergen-specific IgE increased significantly after SLIT, the difference at baseline and the end of the study between the SLIT and placebo groups were not significant. There was, however, a marked, significant increase in mite-specific IgG4 levels with SLIT, and the IgG4/IgE ratios were significantly higher after treatment with SLIT compared with placebo. A significant increase in IgG4 after 18 months of therapy has been previously demonstrated,[10] but we found an increase after only 6 months. The relationship between the duration of SLIT and an IgG4 response require further investigation. The significant increase of IgG4 in our study is further evidence substantiating the effect of SLIT on the immune system over a short treatment period. IgG4 may act as blocking antibodies by engaging low-affinity Fc receptors for immunoglobulins expressed by B lymphocyte, basophils or mast cells.[14] Kolbe *et al.*[15] demonstrated that the blocking antibody activity is dependent on the dose of antigen administered. In our study, the immunologic changes may be attributable to the high dose of mite allergens. Mothes *et al.*[16] showed that these blocking antibodies may have protective effects by inhibiting immediate-type reactions and systemic increases of IgE responses. The importance of an increase in allergen-specific IgG during the course of immunotherapy has been stressed by Flicker and Valenta,[17,18] and a number of studies have found that an increase in IgG4 is important for symptom relief.[19-22] *In vitro* studies have shown IgG4 to inhibit histamine release[20] and IgE-mediated allergen presentation.[23] The timing and clinical role of an increase in IgG4 antibodies is unclear. Despite significant increases in the mite-specific IgG4 levels in children receiving SLIT, we were unable to demonstrate a clear clinical effect over a relatively short 6-month period.

The IgG4/IgE ratios at the end of the study were significantly higher in the SLIT group, similar to changes in the ratio that have been observed in other studies.[11,24,25] The change in the IgG4/IgE ratio has correlated with significant differences of the late-phase skin response as well as with increases in allergen-specific IgG4.[14,26]

The safety profile of Staloral is encouraging. We used an accelerated schedule for dose increases in this study, but there were still no serious systemic reactions. Other investigators have noted mild adverse events involving the buccal cavity (local swelling, reddening, numbness, and tingling of the tongue, buccal mucosa and/or gingiva) and gastrointestinal tract with SLIT,[9,27] but the treatment is generally quite well tolerated.

While we were unable to demonstrate significant improvement in either symptom scores or use of medications, our study did demonstrate that Staloral given for 6 months is associated with a significant increase in mite-specific IgG4 antibodies in Taiwanese children with allergic rhinitis. Given the safety and ease of administration of SLIT, one would hope that manipulation of the treatment duration or dose or both will eventually yield a reliable, effective regimen. The serologic changes induced by the treatment suggest that real changes are occurring. It remains to be seen if this will eventually translate into a clinically effective means of desensitizing children with allergic rhinitis.

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