

A Double-Blind Placebo-Controlled Evaluation of Sublingual Immunotherapy with a Standardized Ragweed Extract in Patients with Seasonal Rhinitis

Evidence for a Dose-Response Relationship

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Key Words

Sublingual-swallow immunotherapy · Ragweed · Seasonal rhinitis

Abstract

Background: There is a growing consensus on the benefits of sublingual-swallow immunotherapy in the treatment of allergic diseases. **Methods:** This randomized, double-blind placebo-controlled study was undertaken to assess the efficacy and safety of sublingual immunotherapy with standardized ragweed pollen extract tablets, in patients with an allergic rhinitis. A total of 110 outpatients were randomized (immunotherapy [I]: 55; placebo [P]: 55), of whom 99 were analyzable for efficacy (I: 48; P: 51) and 106 analyzable for safety (I: 53; P: 53). After a 28-day progression phase, the patients received a maintenance treatment during 6.5 months. Efficacy variables included a global assessment of efficacy (patient/investigator), symptoms and medication scores as well as the frequency of asthma attacks. **Results:** In the active treatment group, 43 patients completed the study, versus 49

on placebo. During the whole period of pollination, the difference favoring immunotherapy was highly significant for the global assessment by the patient ($p = 0.004$) and by the investigator ($p = 0.005$). Adverse reactions were reported more often in the active treatment but mild or moderate, and they abated after dose adjustment. A subgroup analysis of those patients receiving the highest dose of immunotherapy (3 tablets 3 times a week) showed a highly significant response for rhinitis and conjunctivitis total scores by comparison to lower dosages. **Conclusion:** This study confirms the efficacy and safety of sublingual immunotherapy and strongly suggests a dose-response relationship.

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Introduction

Immunotherapy has been used extensively since 1911 as a treatment for allergy [1]. Controlled studies have shown the efficacy of subcutaneous injections with pollen or mite extracts to treat allergic rhinitis and conjunctivitis

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as well as allergic asthma [2]. However, the risks of systemic reaction, sometimes severe, are well known, whereas the need for repeated injections in the doctor's office raises obvious problems of acceptability for the patients. This is why other routes of administration have been investigated, with the overall aim of improving the safety and convenience of immunotherapy. According to a recent World Health Organization (WHO) Position Paper concerning effective treatments for allergic diseases, the sublingual route gave some evidence of efficacy [3, 4]. In birch pollinosis, both SLIT and SCIT have been shown to be effective compared with placebo and without significant difference between the two treatments. SLIT is favored by an advantageous safety profile [5]. However, this route for administering immunotherapy deserves more investigation, and this was the rationale of the present study.

Although a number of studies [6–11] showed a satisfactory benefit/risk ratio for the sublingual-swallow immunotherapy (SLIT) in pollen allergy, the drops used for administration may raise a problem of acceptability as they must be kept under the tongue for 2 min before being swallowed, which is not necessarily easy as the volume administered generally goes up to 20 drops (i.e. 1 ml) per dosing. It appears therefore that availability of a solid form (tablet) allowing progressive sublingual disintegration could represent a progress in terms of patients' acceptability and easiness to use their anti-allergic treatment.

The pollen chosen to test a new form of solid SLIT was ragweed, a widespread cause of allergy in the US. In France, the distribution of this plant is mainly located in the area of the river Rhône.

This double-blind placebo-controlled study was undertaken to assess versus placebo the efficacy and safety of SLIT with a standardized ragweed pollen extract in a solid form administered by the sublingual route, in patients with an allergic rhinitis (possibly associated with symptoms of asthma) caused by ragweed pollen. In addition, the clinical response should be assessed depending on the cumulated administered dose of allergen.

Material and Methods

Study Design

This study was a randomized double-blind placebo-controlled trial on two parallel groups of paediatric or adult patients, carried out in the southeast of France. Throughout the trial, five regular visits were scheduled every 2 months for efficacy and safety assessments; in addition, the patients were requested to fulfil a diary card on a daily basis.

Inclusion criteria were an age between 7 and 55 years, an allergy to ragweed pollen as assessed by a clinical history (seasonal allergic rhinitis), positive skin-prick tests and specific IgE (positive RAST class II and above). Excluded patients were those with symptomatic polysensitizations, asthma not controlled by β_2 -agonists, current treatment with long-acting antihistamine agents, β -blocking agents (even topical), inhaled or oral corticosteroids, cromones.

Primary efficacy variables included symptom and medication scores (daily recorded in the patients' diary card); secondary variables included frequency of asthma attacks, a global assessment of efficacy (patient/investigator), immunological measurements (IgE, IgG4) and skin-prick tests. The safety of the treatment was assessed on the basis of adverse effects.

All patients or their parents should have given written informed consent. The study was conducted in accordance with the Good Clinical Practices after approval by the Lyon ethics committee and the French health authority.

Treatments

The active treatment was a standardized ragweed extract (Stallergènes SA, Antony, France), in the form of drops (progression phase) and of tablets. The biological unit used to label extracts was the index of reactivity (IR); an in-house reference extract (labelled 100 IR) was defined as the concentration eliciting a wheal of 7 mm mean diameter in 30 skin-prick-tested allergic patients. The in-house reference standard of ragweed extract contained 70 μ g Amb a1 major allergen. Three concentrations of the allergen were used: 1, 10 and 100 IR/ml. The placebo was first a solution and then tablets, each of them with an appearance similar to that of the active agent administered within the same time.

The treatments were administered sublingually from March to October; the drops were administered directly or put on a piece of sugar or bread which was held under the tongue for 2 min before being swallowed, whereas the tablets were held under the tongue until they disintegrated completely. 1 ml of 100 IR solution and one 100 IR tablet contained 160 μ g Amb a1 major allergen.

The treatment schedule included a progression (28 days), a transition (30 days) and a maintenance phase (5.5 months) during the whole of the pollen season. The progression dose was of 2 drops/day of the lowest concentration (1 IR/ml) up to a maximum 20 drops/day of the highest concentration (100 IR/ml); then the tablets (100 IR/tablet) were introduced in alternation and/or association with the drops in order to reach 300 IR/day. The dose of 3 tablets/day (300 IR) was maintained during the transition phase (from day 28 to day 58) and followed by a maintenance dose of 1, 2 or 3 tablets 3 times a week depending on the patient's tolerance. Therefore, the cumulative dose received by each patient throughout the study was variable.

Allowed drugs, taken as necessary, included ocular (levocabastine) and oral (ceterizine) short-acting anti-histamine agents, topical (flunisolide) or oral (prednisolone) corticosteroids, as well as salbutamol in asthmatic patients.

Study Assessments

Daily control was based upon recording by the patients (or their parents) in a diary card of:

- A symptom score according to a 0–3-grade scale (0 = no symptom; 1 = mild; 2 = moderate; 3 = severe) for rhinitis (sneezing, rhinorrhea, nasal blockage, pruritus) and conjunctivitis (itching, redness or weeping).

- Their consumption of allowed antiallergic drugs: computer-based weighting for each drug according to its dose. One drop of levocabastine in each eye, one puff of flunisolide in each nostril, one puff of salbutamol or 1 mg prednisolone were scored 1. One tablet of ceterizine was scored 2.

At the end of the study, both patient and investigator were requested to record their global assessment of the efficacy of the immunotherapy, according to a 4-grade scale (1 = success; 2 = partial improvement; 3 = no change; 4 = worsening).

Skin tests were performed at the beginning and at the end of the study, in duplicate on the patients' forearms, with ragweed extract at five dilutions (500, 167, 56, 19 and 6 IR/ml). Skin reactions were recorded after 20 min and, after tracing on a transparent tape, measured with a scanner to establish a dose-response curve for each patient.

Immunological measurements included allergen-specific IgE and allergen-specific IgG4 and were performed at the beginning and end of the study. After sampling, the assays were made in a central laboratory.

Throughout the trial, adverse events were recorded on the basis of clinical examination (on regular visits) as well as of the patient's spontaneous complaints. In addition, the evolution of the pollen grain counts was recorded. As ragweed exists in France in a fairly narrow area, possible travelling of the patients outside this area was taken into consideration in the statistical analysis.

Statistical Analysis

Qualitative variables were described by absolute and relative frequencies, with between-group comparisons using χ^2 or Fisher's exact tests. Quantitative variables were described by arithmetic mean, standard deviation (SD), standard error of the mean (SEM), observed range, with between-group comparisons using Student's *t* test. All tests were bilateral, with an α -level = 0.05. The patients were also compared with patients receiving the highest dose permitted (per protocol versus intent-to-treat analysis).

Results

Patients, Dose Administered and Exposure to Pollen

A total of 110 patients were included in the 11 centers of the trial; of them, 55 were randomized in the active treatment group and 55 in the placebo group. Prior to the treatment initiation, 4 patients withdrew (2 in each group) due to withdrawal of consent: they were not assessable. Seven additional patients withdrew prior to the pollen season (5 in the active treatment group and 2 in the placebo group): they were not assessed for efficacy, but remained within the population analyzed for safety (*n* = 106). Of the remaining 99 patients assessable for efficacy, 48 received the active drug and 51 a placebo. Of them, 7 additional patients stopped their treatment prior to normal completion of the study (5 in the active treatment group and 2 on placebo), whereas 90 patients remained on treatment until the study completion (43 in the active treatment group and 49 on placebo). Overall, the percent-

Table 1. Demographic characteristics and medical history of the patients randomised into the trial (*n* = 110)

Characteristics	Active treatment (<i>n</i> = 55)	Placebo (<i>n</i> = 55)
Sex		
Male	22 (40%)	23 (41.8%)
Female	33 (60%)	32 (58.2%)
Age, years		
Mean $\pm$ SD	35.1 $\pm$ 8.4	34.7 $\pm$ 10.65
Range	18–53	8–62
Rhinitis duration, years		
Mean $\pm$ SD	7.1 $\pm$ 6.4	7.3 $\pm$ 6.3
Range	0.6–35.5	0.6–32.7
History of conjunctivitis	39 (70.9%)	43 (78.2%)
History of asthma	13 (23.6%)	17 (30.9%)
Drug treatment during previous season	52 (94.6%)	51 (92.7%)
Asymptomatic polysensitization	17 (30.9%)	19 (34.5%)
Skin test – wheal, mm (mean $\pm$ SD)	10.8 $\pm$ 3.9	10.5 $\pm$ 3.7
Specific IgE to ragweed RAST (mean $\pm$ SD)	24 $\pm$ 77	18 $\pm$ 46

Table 2. Cumulated dose (IR) administered throughout the study

Form	Active treatment (<i>n</i> = 55)	Placebo (<i>n</i> = 55)
Solution		
Mean $\pm$ SD	970 $\pm$ 14,098	613 $\pm$ 44.6
Range	326–8,201	426–738
Tablets*		
Mean $\pm$ SD	21,948 $\pm$ 9,221.8	28,865 $\pm$ 2,795.7
Range	1,300–30,500	15,300–30,500

* *p* < 0.0001.

age of patients withdrawn prematurely differed significantly (*p* = 0.04) between the active treatment (14/55, i.e. 25.5%) and the placebo group (6/55, i.e. 10.9%).

The main demographic characteristics of the randomized patients and their medical history are summarized in table 1; there was no significant difference between both treatment groups for these items. There was no major difference between the population of included patients (*n* = 110) and that of the patients analyzed for efficacy (*n* = 99).

Recorded on entry, i.e. prior to the pollen season, the baseline medical characteristics showed that allergic

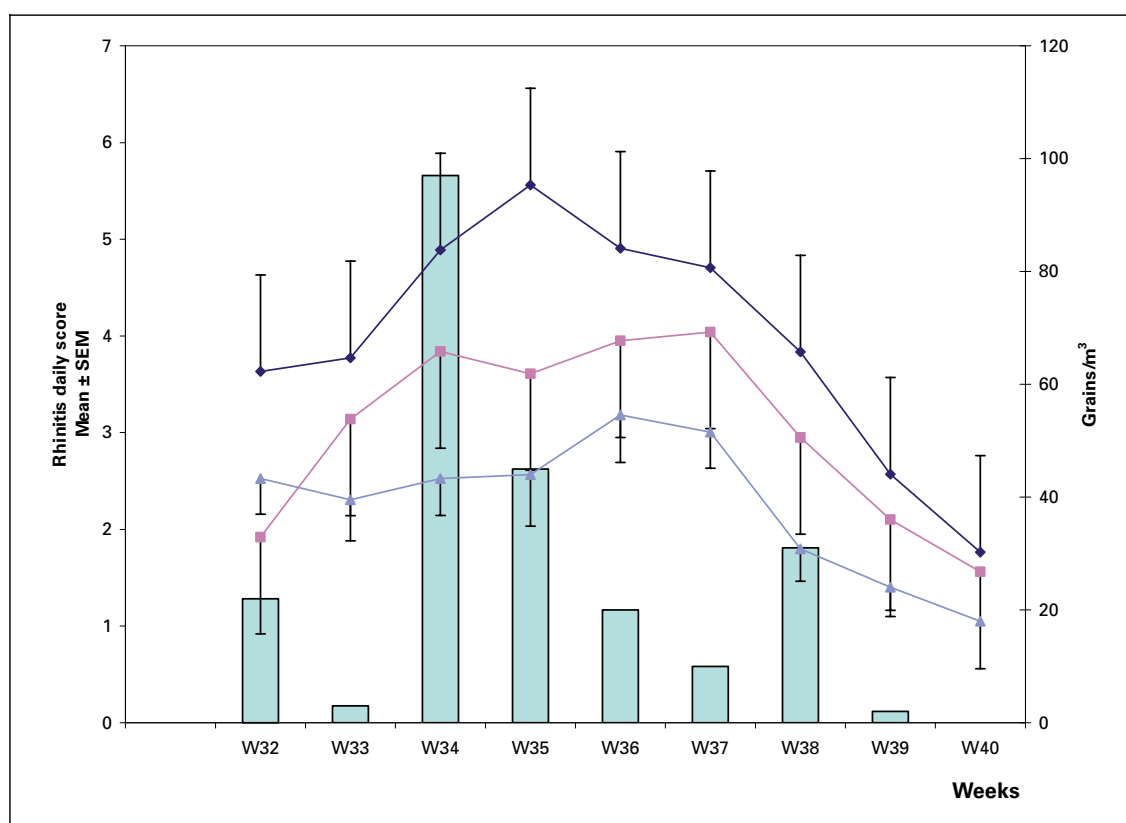


Fig. 1. Mean rhinitis daily score of actively treated and placebo patients. Observation weeks are shown on the x-axis. Scores (left y-axis) are indicated by triangles for patients treated with 3 ragweed 100 IR tablets 3 times a week, by lozenges for patients treated with a 1 or 2 ragweed tablets 3 times a week and by squares for those on placebo. Pollen count (right y-axis) is given as bars corresponding to daily mean.

symptoms were almost absent (rhinitis $\leq 7\%$; conjunctivitis $\leq 3\%$), whereas the consumption of anti-allergic drugs was quite low. There was no significant difference between both groups for the baseline symptoms of rhinitis, conjunctivitis and asthma, as well as the medication scores.

The cumulated doses (in IR) administered during the study are given in table 2. The difference between the immunotherapy and the placebo groups was significant for the tablets, as a consequence of dose reductions or interruption in the active-treatment group due to adverse reactions. During the maintenance phase, the percentage of the patients in the active-treatment group who took 1, 2 or 3 tablets 3 times a week was, respectively, 16.7, 20.8 and 54.2%; 8.3% went on taking the solution form. In the placebo group, almost all the patients (94.1%) reached the maximum dose of 3 tablets 3 times a week.

An analysis was performed on the subgroup of 26 patients (54% of the patients on immunotherapy and

assessable for efficacy) who received the highest dose (3 tablets three times a week) during the maintenance period; this subgroup was compared for efficacy variables with the patients of the placebo group assessable for efficacy and the patients treated with a reduced dosage. As regards demographic and baseline variables (sex, age, duration of the disease, skin reactivity, IgE levels), these subgroups were comparable on entry into the trial.

The pollen season occurred between weeks 32 and 40. The timing of pollinization is shown in figures 1–3.

Efficacy Results

Efficacy Variables

At the end of the study, the global assessment of efficacy by the patients was largely consistent with that of investigators and both showed a high statistical significance, for the listed categories as a whole, favoring immunotherapy (table 3). There was no significant difference between both treatment groups for the symptom and medication

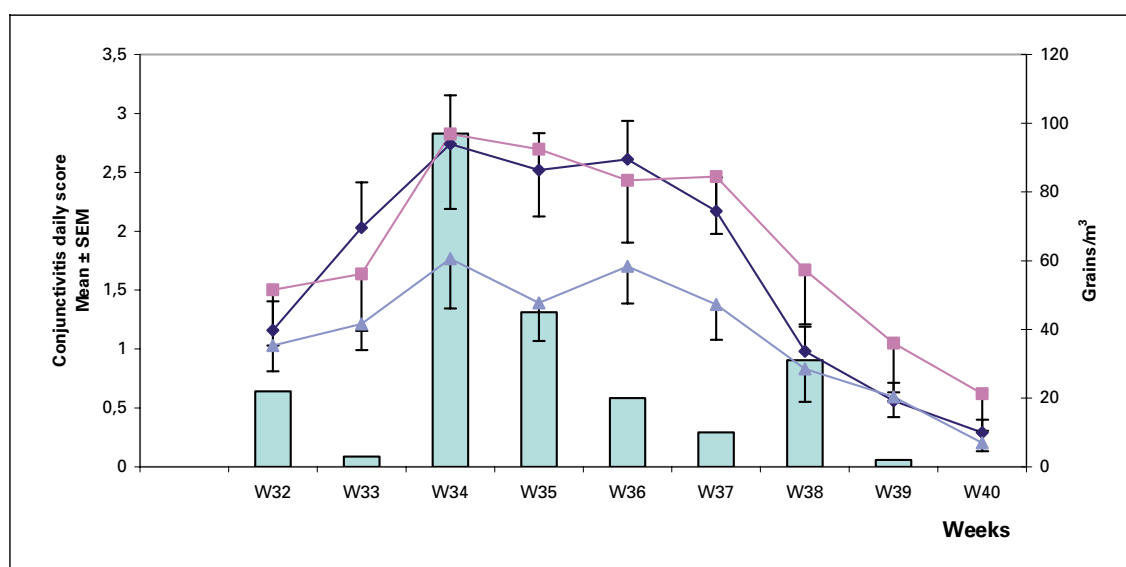


Fig. 2. Mean conjunctivitis daily score of actively treated and placebo patients. Observation weeks are shown on the x-axis. Scores (left y-axis) are indicated by triangles for patients treated with 3 ragweed 100 IR tablets 3 times a week, by lozenges for patients treated with a 1 or 2 ragweed tablets 3 times a week and by squares for those on placebo. Pollen count (right y-axis) is given as bars corresponding to daily mean.

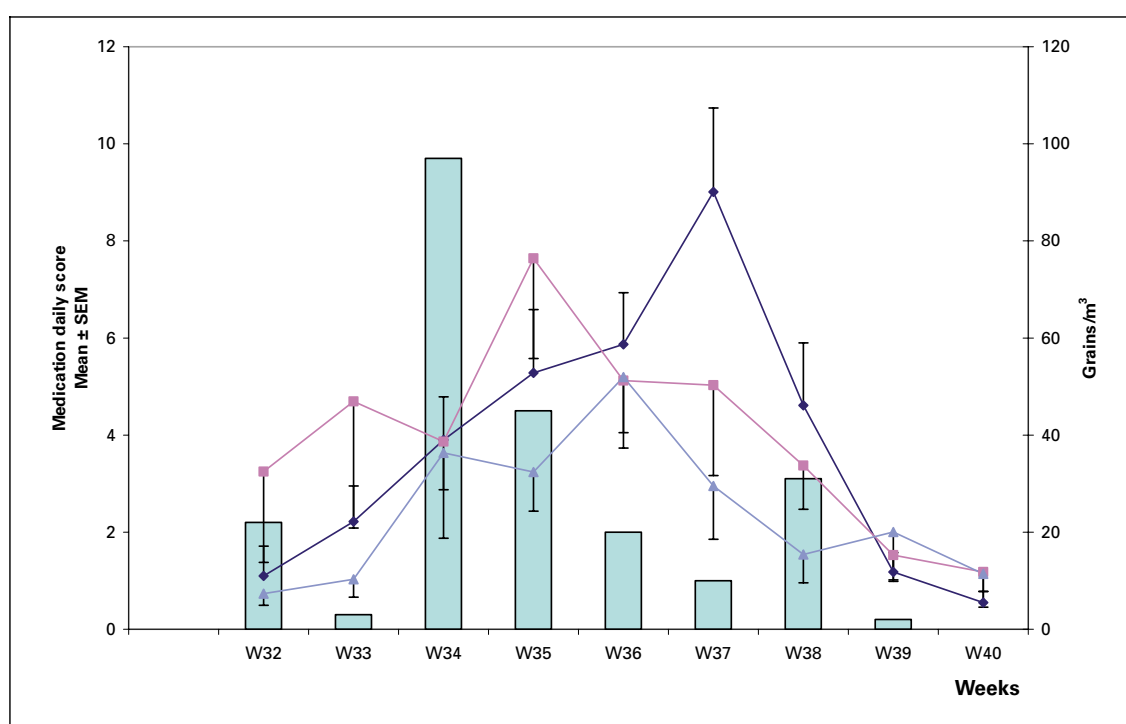


Fig. 3. Mean medication daily score of actively treated and placebo patients. Observation weeks are shown on the x-axis. Scores (left y-axis) are indicated by triangles for patients treated with 3 ragweed 100 IR tablets 3 times a week, by lozenges for patients treated with a 1 or 2 ragweed tablets 3 times a week and by squares for those on placebo. Pollen count (right y-axis) is given as bars corresponding to daily mean.

Table 3. Global assessment of efficacy by the patient and by the investigator at the end of the study

Grade	Patient				Investigator			
	active treatment		placebo		active treatment		placebo	
	n	%	n	%	n	%	n	%
Success	22	47	12	24	22	47	12	24
Partial improv.	19	40	21	42	16	34	17	34
No change	6	13	16	32	9	19	20	40
Worsening		0	1	2		0	1	2
p			0.004				0.005	

scores calculated during the whole of the pollen season (weeks 32–40), although most scores were consistently lower in the active-treatment as compared to the placebo group. The percentage of patients experiencing asthma attacks during the pollen season was 8/47 on immunotherapy (17%) versus 17/51 (33.3%) and this difference was of marginal statistical significance ($p = 0.064$).

Statistical analysis of the efficacy parameters of the 26 patients receiving 3 tablets three times a week showed that the differences consistently favored immunotherapy, reaching statistical significance for a number of them: sneezing, rhinorrhea, total score for rhinitis, weeping, total score for conjunctivitis. As far as medications are concerned, statistical significance was not reached for the total score, but the consumption of each drug was low in both groups on the whole with a tendency consistently in favor of immunotherapy, reaching significance ($p = 0.05$) for corticosteroids (table 4).

Whereas the main baseline characteristics of the patients receiving the highest dose of immunotherapy (3 tablets 3 times a week) during the maintenance period ($n = 26$) and those receiving inferior doses ($n = 22$) were comparable on average, the therapeutic response was significantly higher in the former subgroup for the following efficacy parameters: sneezing ($p = 0.011$), rhinorrhea ($p = 0.028$), nasal obstruction ($p = 0.038$), total score for rhinitis ($p = 0.019$), weeping ($p = 0.038$) as well as ceterizine consumption ($p = 0.028$). For the other efficacy parameters, there was a tendency favoring the highest dose. In the comparison between the patients receiving less than 3 tablets 3 times a week and the placebo group no significant variation was observed. Evolution of total scores for rhinitis, conjunctivitis and medications is presented in figures 1–3.

Skin Tests

Overall, skin-prick tests showed a marked reduction in skin reactivity (in terms of wheal and erythema diameter)

Table 4. Efficacy parameters (daily mean over the pollen period) in the patients of the immunotherapy group receiving the highest dose ($n = 26$) as compared to patients receiving placebo ($n = 48$)

Symptom	Active treatment	Placebo	p
<i>Rhinitis</i>			
Sneezing			
Mean ± SD	0.63±0.34	0.87±0.58	0.03
Range	0–1.27	0–2.62	
Rhinorrhea			
Mean ± SD	0.65±0.44	0.95±0.58	0.02
Range	0–1.62	0–2.57	
Nasal blockage			
Mean ± SD	0.55±0.45	0.78±0.61	0.09
Range	0–1.87	0–2.67	
Nasal puritus			
Mean ± SD	0.46±0.46	0.51±0.59	0.72
Range	0–1.34	0–2.19	
Total rhinitis score			
Mean ± SD	2.27±1.42	3.09±2.14	0.05
Range	0–5.52	0–10.05	
<i>Conjunctivitis</i>			
Itching			
Mean ± SD	0.51±0.46	0.67±0.53	0.21
Range	0–1.61	0–2.67	
Redness			
Mean ± SD	0.35±0.36	0.52±0.52	0.10
Range	0–1.28	0–2.62	
Weeping			
Mean ± SD	0.25±0.24	0.49±0.49	0.01
Range	0–0.85	0–2.24	
Total conjunctivitis score			
Mean ± SD	1.11±0.91	1.69±1.48	0.04
Range	0–3.43	0–7.52	
<i>Medication</i>			
Total medication score			
Mean ± SD	2.41±3.09	4.00±4.24	0.09
Range	0–12.00	0–14.78	

Table 5. Adverse events reported during the study

Description	Active treatment (n = 53)	Placebo treatment (n = 53)
Buccal burning/pruritus	19	0
Bucco-lingual edema	18	0
Gastrointestinal event	16	3
Labial edema	3	0
Asthma (related to the study treatment)	3	2
Other	8	6

of the patients receiving immunotherapy, and the differences with placebo reach statistical significance ($p < 0.03$) for the quantitative test at the dilutions of 6 IR, 19 IR and 167 IR.

Immunological measurements IgE levels increased more in patients on immunotherapy (+51%) than in the placebo (+30%) group ($p = 0.0005$), and this was also true for specific IgG4 levels with a respective increase of 56 versus 7% ($p < 0.0001$).

Adverse Events

No serious adverse events were reported during the study. Adverse events leading to treatment discontinuation were reported in 4 patients of the active treatment group (sublingual burning, oral pruritus with vomiting and headache, pruritus and lingual oedema with gastralgia and diarrhea, asthma and gastralgia) and in one patient receiving placebo (gastralgia).

Overall, adverse events were more frequent in the patients receiving immunotherapy (70%) than in those of the placebo group (13%), the difference being significant ($p < 0.0001$) (table 5). Most of the events (86%) reported in the immunotherapy group were assessed as 'probably' related to the study treatment versus 33% in the placebo group. Most adverse events were of mild or moderate intensity and were reported at the beginning of the study and did not persist after dose adjustment.

Discussion

The design of the present study complied with the most recent recommendations in this regard [2], with a focus on symptom scores and medication consumption as primary efficacy variables.

As expected, allergic symptoms were mild and almost absent on entry into the trial. During the pollen season, statistical analysis showed a very consistent effect of immunotherapy on all the primary and efficacy parameters, reaching significance for global assessments (by the patient and by the investigator) and objective parameters (skin reactivity, and levels of IgE or IgG4). Of marginal statistical significance, the favorable effect of the active treatment on the number of days with asthma concerns one of the most severe potential complications of allergy.

In spite of the large cumulative doses administered (22,918 IR containing 36.7 mg Amb a 1 in the active treatment group over the whole of the study), the treatment appeared safe, without any suggestion of severe anaphylaxis and nor evidence of respiratory reactions specific of active treatment as compared to placebo. Adverse events recorded during this study were more frequent with active treatment than with placebo, but most of them were mild or moderate and tended to occur at the beginning of the study, reflecting the need for dose adjustment on an individual basis.

Although the experimental design was not that of a dose-response study, exploratory statistical analyses strongly support a dose-response relationship, those patients receiving the highest dose permitted by the protocol (3 tablets 3 times a week during the maintenance period) showing significant responses as compared to placebo on a number of primary efficacy parameters (total score for rhinitis and for conjunctivitis in particular) whereas the responses appeared also significantly better when compared to lower doses of the active treatment. A similar dose-effect relationship was already demonstrated many years ago for injection immunotherapy by venoms [12], pollens [13, 14] and mites [15]. This applies to the curative effect of treatment on symptoms but also to the preventive effects on polysensitization and asthma. These effects were only obtained with high doses. Recent studies have confirmed these data [16–18].

The superior efficacy of high doses of SLIT could be related to the role of Langerhans cells. An *in vitro* study has clearly demonstrated the dose- and time-dependency of allergen internalization by dendritic cells [19].

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