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Sublingual immunotherapy: a double-blind, placebo- controlled trial with *Parietaria judaica* extract standardized in mass units in patients with rhinoconjunctivitis, asthma, or both

Key words: clinical efficacy; *Parietaria* allergy; placebo-controlled study; sublingual immunotherapy.

Background: New routes of administering immunotherapy in respiratory allergy are being studied as an alternative to conventional injective immunotherapy. We carried out a study to evaluate the clinical efficacy and effects of sublingual immunotherapy in patients with *Parietaria judaica*-induced respiratory allergy.

Methods: A double-blind, placebo-controlled design was followed. Thirty patients with *P. judaica* rhinoconjunctivitis, mild asthma, or both were randomly chosen for sublingual immunotherapy (14 patients) or placebo treatment (16 patients). The patients underwent preseasonal rush induction treatment followed by coseasonal maintenance treatment during the *Parietaria* pollen season. Symptom and drug scores, as well as specific IgE and specific IgG4, were recorded.

Results: Significantly lower symptom and drug scores were found ($P=0.04$), especially during the *Parietaria* pollination period, in the immunotherapy group. No significant difference in specific IgE and specific IgG4 was detected between the active and placebo groups; a statistically significant increase of specific IgE was detected in both groups ($P=0.05$). No patient undergoing active sublingual immunotherapy reported local or systemic side-effects.

Conclusions: Our data suggest that sublingual immunotherapy is both clinically effective and safe in treating patients with *Parietaria*-induced rhinoconjunctivitis and mild asthma.

At present, specific immunotherapy is the only treatment known that can affect the natural course of respiratory allergic diseases by modifying the immune response (1). Conventional injective immunotherapy has been shown to

be clinically effective if diagnosis, administration, and patient monitoring are carried out correctly (2), but its safety and patient compliance remain to be defined. Therefore, experimental and clinical efforts are underway both to improve allergen standardization and to explore new routes of immunotherapy administration (3).

The most interesting alternative route of immunotherapy administration, the subject of several studies, is the sublingual one or, rather, the sublingual/swallow method (4–15). Its safety and efficacy have only recently been recognized in a WHO position paper (5), on the basis of four double-blind, placebo-controlled studies selected according to strict criteria such as minimum number of patients, amount of allergen extract administered, and duration of treatment (8, 11, 13, 14). The EAACI position paper on local immunotherapy published more recently (17) reached the same conclusions on the basis of two further studies (18, 19).

As a result of these encouraging reports and the data we had previously obtained with an open rush sublingual immunotherapy (SLIT) trial with *Parietaria judaica* extract (16), we undertook a double-blind, placebo-controlled trial to assess the efficacy of *P. judaica* SLIT, using a highly standardized allergen extract.

The *P. judaica* allergen was chosen, as it is widespread in the Mediterranean area over a long pollen season (February to October). The frequency of sensitization to this pollen is very high: statistics show an incidence of 10–60% along the Mediterranean coast of Spain; approximately 25% in the Mediterranean area of France; and, in Italy, frequencies of 30–60% in central Italy, around 70% in Liguria (20), and up to 80% in Sicily (21).

Material and methods

Selection of patients

Thirty subjects (14 men and 16 women) were enrolled in this study between June and December 1995. All patients had to have a positive history for rhinitis, asthma, or both for at least 2 years during the *Parietaria* pollen season. The patients were randomly separated into two groups for placebo or active treatment; the characteristics of the two groups of patients were similar in mean age, sex, and allergic disease (Table 1).

Subjects were enrolled if they had had symptoms of allergic rhinoconjunctivitis with or without moderate asthma (FEV₁ values of 70–90% of predicted values) for at least 2 years during the *Parietaria* pollen season and a skin

Table 1. Demographic data of patients enrolled in trial

Therapy	Age (years)	Sex	Disorder
Active	32 ± 17	7 M+7 F	7 RC+ 7 RCA
Placebo	32 ± 18	7 M+9 F	8 RC+8 RCA
R: rhinitis; C: conjunctivitis; A: asthma.			

prick test positive for the *P. judaica* extract resulting only in a wheal greater than or equal to that obtained with 10 mg/ml histamine. The *P. judaica* allergen used for the prick test had been purified and biologically standardized according to the BU protocol developed by ALK-Abelló SA, Madrid, Spain, with a potency of 100 BU/ml (22). This biologic potency corresponded also to a content of about 6 µg/ml of *P. judaica* major allergen (Par j 1) according to the producer's quality control methods (23). Specific IgE to *P. judaica* levels in sera were determined by the RAST EIA technique (Pharmacia Diagnostics AB, Uppsala, Sweden), and only patients showing at least a class 3 result were selected for the trial. Exclusion criteria were severe asthma (FEV₁ values below 70% of predicted values), previous specific immunotherapy, sensitization to other inhalant allergens, pregnancy, or other general contraindications for immunotherapy as established in the EAACI position paper (1).

Other treatment, especially long-acting antihistamines and cutaneous topical or systemic corticosteroids, was prohibited during the study. However, rescue treatment was authorized in the form of antihistamines tablets (cetirizine 10 mg), nasal topical steroids (beclomethasone dipropionate 20 mg), and inhaled β₂-adrenergic agonists (salbutamol 40 mg).

Allergen-specific treatment

The sublingual dose used throughout the study was prepared from the same batch of freeze-dried *P. judaica* extract and was identical to the commercial product. The therapy consisted of five 3-ml vials with a concentration of 0.016 BU/ml (vial 0), 0.08 (vial 1), 0.04 (vial 2), 2 (vial 3), and 10 (vial 4) in physiologic saline with 50% v/v of glycerol and 0.4% w/v of phenol. The major allergen Par j 1 content in the vial with the maximum concentration was 0.6 µg/ml.

Placebo preparations were identical to the active therapy in composition, appearance, presentation, taste, and color, but obviously contained no allergen. The treatment schedule was started in January, before the beginning of the *P. judaica* pollination period, after a 2-week rush-inducing schedule. The drops were to be taken from the different

vials, and had to be taken twice daily, starting with one drop from vial 0 and increasing by one drop at each administration up to a dose of five drops of the same vial and then repeating the procedure with the subsequent vials. The maximum dose of five drops from vial 4 was then administered three times a week. This maintenance dosage was continued until the end of September.

Each dose had to be taken in the morning on an empty stomach; the drops of allergen had to be kept in the mouth for at least 2 min without swallowing, and then any remaining liquid had to be spit out. According to the schedule, by the end of the trial, an average cumulative dose of 199.5 BU of allergen had been administered to each patient in the active sublingual therapy group, equal to 12.77 µg of Par j 1.

Control parameters

Skin reactivity

Duplicate prick tests, with increasing concentrations of Par j 1 extract of 5, 20, 100, and 500 BU/ml, respectively, were performed before the beginning of the SLIT (To, December 1995) and at the end of the trial (T1, October 1996).

Pollen counts

P. judaica pollen counts for the 1996 season were obtained from the aerobiology station of the University Hospital of Messina. The pollen trap (VPPS 2000, Lanzoni, Bologna, Italy) was located on the roof of a 15-m-high building.

Monitoring of patients

All patients were instructed to keep a diary from the beginning of February to the end of September for the daily evaluation of symptoms. Nasal symptoms (itching, sneezing, nasal discharge, and nasal obstruction), eye symptoms (itching and redness), coughing, symptoms of bronchial obstruction, and wheezing had to be reported according to a 0–3 grading (0=no symptoms, 3=severe symptoms). The patients had to record the use of drugs, and a specific daily score was given (1 point for each topical drug administration, 2 points for each cetirizine tablet).

Immunologic parameters

P. judaica-specific IgE and *P. judaica*-specific IgG4 were determined at the end of the trial by means of commercial kits according to the supplier’s instructions (CAP System,

EIA method, Pharmacia AB, Uppsala, Sweden) in samples of sera taken at To and T1 and stored frozen.

Missing data

Aerobiologic data were not available on two days (13 and 14 May) in the monitoring period. The figures for 12 and 15 May were averaged to supply the missing data from the pollen count graph.

Statistical analysis

Symptom and drug-intake data were statistically analyzed by the Mann–Whitney U-test (BMDP Statistical software, Cork, Ireland). *P. judaica*-specific IgE and *P. judaica*-specific IgG4 and wheal areas of prick tests were statistically analyzed by parametric tests (ANOVA), after the data had been checked for normal distribution.

Ethics

The protocol under which this study was conducted was approved by the ethics committee of the University Hospital of Messina. In addition, the Italian Minister of Health was notified. All subjects signed informed consent statements before participation in the study.

Results

Symptom and medication scores

The symptom scores for conjunctivitis, rhinitis, and asthma were significantly lower in the active-treatment group ($P=0.04$), and medication consumption was also signifi-

Table 2. Mean values and statistics of immunoglobulins before and after treatment (ANOVA)

Immunoglobulins		Average ± SD		
		T0	T1	T0–T1
Active	IgE	14.058 ± 14.136 (SEM=3.9207)	19.304 ± 24.763 (SEM=6.8681)	0.0277
	IgG4	2.52 ± 0.333 (SEM=0.092)	2.57 ± 0.411 (SEM=0.114)	P=0.7798 (NS)
Placebo	IgE	17.42 ± 13.14 (SEM=3.2860)	22.19 ± 20.295 (SEM=5.0738)	P=0.0340
	IgG4	2.6637 ± 0.6394 (SEM=0.1599)	2.5850 ± 0.7040 (SEM=0.1760)	P=0.3519 (NS)

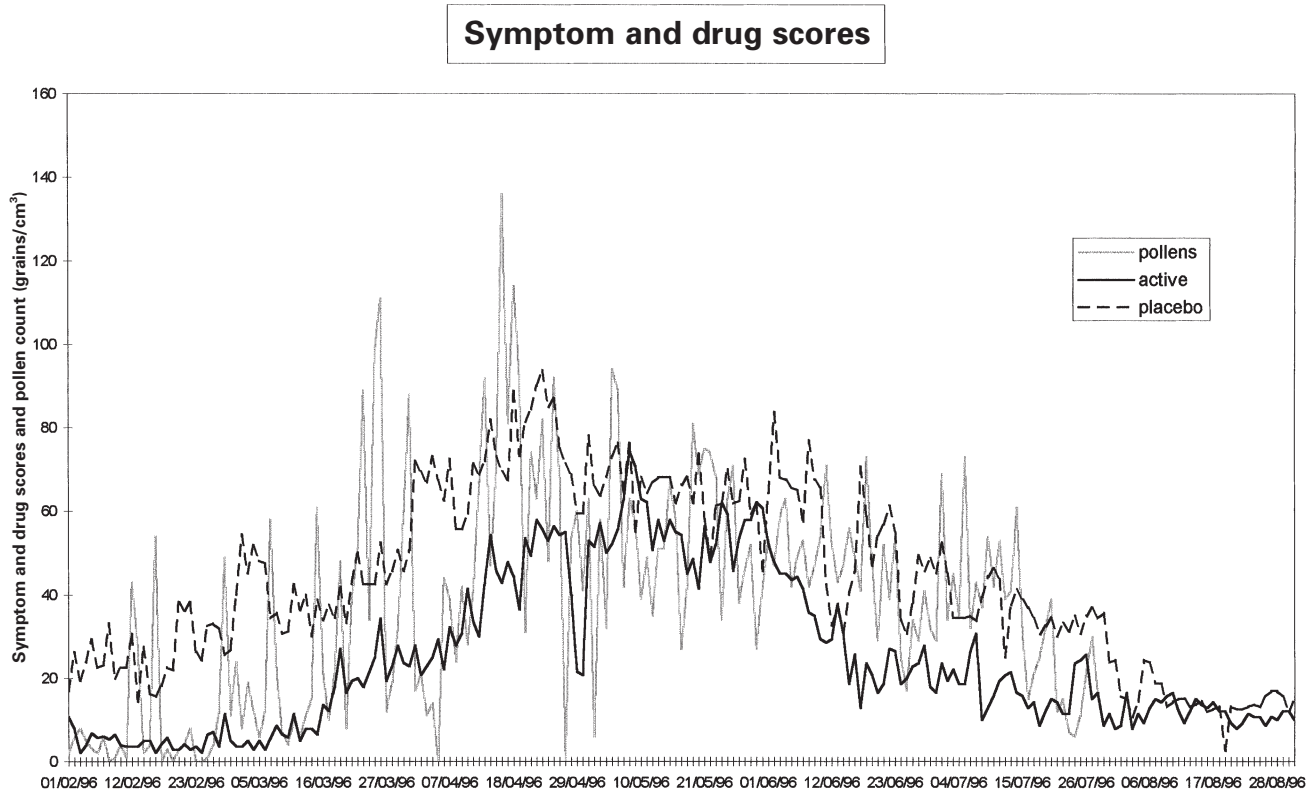


Figure 1. Drug and symptom scores of patients (average daily score) in relation to *Parietaria* pollen counts.

cantly less ($P=0.05$). There was also a significant difference in rhinoconjunctivitis and asthma scores between the two groups ($P=0.001$). Comparison of the drug-plus-symptom scores between treated and placebo groups showed a reduction of over 45% in favor of the active group, a statistically significant difference ($P=0.04$), in spite of the reduced number of patients enrolled, especially in the peak pollen season between April and June (Fig. 1). If drugs alone are considered, there was a 60% reduction in the active group ($P=0.05$).

Objective parameters

The titrated prick tests with *P. judaica* extract did not differ significantly from To to T1. No significant variation of specific *P. judaica* IgE and specific *P. judaica* IgG4 was detected between the active and placebo groups (Table 2). A statistically significant increase in specific IgE ($P<0.05$) was detected in both the active and placebo groups from To to T1.

Side-effects

All patients completed the study, and no local or systemic side-effects were reported by either group.

Discussion

In a recent review on SLIT, Malling (4) pointed out that further studies were required before this alternative specific treatment could be recommended for routine use, since its efficacy and safety had been insufficiently documented, and existing reports concerned only mite, *Parietaria*, and grass allergens. The need for further documentation was also underlined in the WHO position paper (5) and in the more recent EAACI position paper on local immunotherapy (17).

One of the most frequently discussed issues is the amount of allergen extract to administer, as the reports in the literature are controversial. Clinical efficacy with SLIT was shown both by Scadding & Brostoff (6), who reported the results obtained with very low-dose SLIT, and Quirino et al. (15), who reported experience with sublingually treated patients who received more than double the cumulative dose (2.4 times higher) given in ordinary injective therapy. In our study, clinical efficacy was reached by administering a *P. judaica* extract standardized in BU (biologic units) to guarantee the consistent composition and biologic potency of the allergen extract, and quantified in MU (mass units) to allow the major allergen to be quantified in $\mu\text{g/ml}$ (24, 25). This enabled us to calculate that within a month the patient

reached a cumulative dose of 24 BU (1.44 µg Par j 1), three times higher than that usually administered by injective immunotherapy over the same period (about 8 BU, equal to 0.46 µg of Par j 1). This high dosage was well tolerated and did not cause any side-effects.

Since, in our trial, the allergen extract for SLIT was retained in the mouth for 2 min and then spit out, without being swallowed, as is the practice with sublingual/swallow immunotherapy, we assume that the allergen absorption occurred mainly through the oral mucous membranes, but we cannot exclude the possibility that some of the allergen could have been swallowed. Using a radiolabeled *P. judaica* allergen, for an exclusively sublingual treatment, Bagnasco et al. (26) have documented, on the one hand, a complete absence of absorption of the radiolabeled allergen through the sublingual mucosa up to 30 min from administration of the allergen extract, and on the other hand, the persistence of a significant amount of allergen at the oral mucosa level for at least 20 h after administration. In this study, the authors suggest that these data may support the hypothesis that a slow absorption and processing of the locally retained allergen occurs through the local oral immune system. In addition, these data suggest that there is little risk of too rapid an absorption of the allergen through the sublingual or oral mucosa.

We observed that the *Parietaria*-specific IgE antibodies increased significantly in both groups, as usually occurs under environmental allergenic pressure, but we were not able to show any significant modification of the skin reactivity or of the IgG4 antibodies, as shown even in

recently published sublingual clinical trials (27), probably because in our study the SLIT treatment lasted 9 months only. This agrees with our previous study (16) and similar studies where the treatment period was less than 12 months (11, 14, 15). However, trials with a treatment period of at least 18 months did show significant changes in some objective parameters (8, 13, 28). In our trial, the patients followed a rapidly inducing schedule of SLIT administration which allowed the maintenance dose to be reached in only 13 days, the treatment being started in January, just before the onset of *P. judaica* pollination (usually in February).

All the patients treated showed an excellent tolerance of the therapy, and the safety of SLIT was also associated with good clinical efficacy; the symptoms and drug scores, especially during the peak of *P. judaica* pollination, showed a statistically significant difference with a reduction of over 45% in patients treated with the active therapy.

Therefore, we can conclude that, according to the results obtained in our trial, SLIT may be considered a valid treatment to be recommended as an alternative to injective therapy for patients who, for various reasons, are unable or not available to start the treatment in October, at the end of the *Parietaria* season.

Nevertheless, we would like to emphasize that it is preferable to administer SLIT to selected patients who offer good compliance with the treatment, and who are willing to cooperate with the allergist and follow the suggested schedule carefully, and even if the therapy is self-administered, the patient should be advised to see the allergist for a periodical examination.

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