

# Controlled Study of Preseasonal Immunotherapy with Grass Pollen Extract in Tablets: Effect on Bronchial Hyperreactivity

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**Summary.** *Background:* Based on experimental results, the sublingual route for immunotherapy (IT) has been accepted as a viable alternative to the injection route, but few data on the effects on asthma are so far available. *Objective:* In the present open controlled trial we evaluated whether a preseasonal IT with grass pollen in orosoluble tablets added to pharmacotherapy, can improve non-specific bronchial hyperreactivity. The clinical efficacy was evaluated as well. *Methods:* Fifty-one patients (mean age 27.4 years) suffering from rhinoconjunctivitis and/or mild-intermittent/mild-persistent asthma due to grass pollen were allocated to two groups receiving pharmacotherapy alone ( $n = 25$ ) or pharmacotherapy plus IT in tablets ( $n = 26$ ). A methacholine test was performed in asthmatic subjects out of the pollen seasons at baseline and after 3 years of treatment. Symptom scores and drug intake were evaluated during pollen seasons by a diary card. *Results:* A significant increase  $p = .01$  in the PD20 at the methacholine test was observed in the IT group compared to the control group. A significant clinical improvement both for rhinitis ( $p = .001$ ) and asthma ( $p = .001$ ) was observed in the IT group, and this improvement was paralleled by a clear-cut reduction of drug intake ( $p = .001$ ). An improvement of rhinitis symptoms without modification of drug intake was seen in the control group ( $p = .01$ ). The treatment was well tolerated and no relevant side effect was reported during the 3 years. *Conclusion:* The investigated local IT reduced the nonspecific bronchial hyperreactivity. Furthermore, it was clinically effective and safe.

**Key words:** local immunotherapy, nonspecific bronchial hyperreactivity, rhinitis, asthma

## Introduction

During recent years the local (non injective) routes for immunotherapy (IT) administration have been extensively investigated [1]; based on the available literature, both WHO [2] and EAACI [3] have accepted the sublingual/swallow route as a viable alternative to the subcutaneous route in adults. Concerning the

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sublingual/swallow IT, a recent pharmacokinetics study [4] conducted in healthy humans showed that both local immunity (oral mucosa) and systemic immunity (swallowed fraction) may be involved in the mechanisms of action of the allergenic extract. Therefore correct administration of an enteral vaccine should involve contact with the oral mucosa. The clinical effectiveness was clear-cut for pollen- and mite-induced rhinoconjunctivitis, whereas some controversies still remain for asthma. In the present study we investigated a local preseasonal IT with grass pollen extract prepared as orosoluble tablets, in a population of patients suffering from asthma and/or rhinoconjunctivitis due to grass pollen. In those patients suffering from asthma, the bronchial non-specific hyperreactivity was evaluated by the methacholine (MCh) test before and after IT. The clinical efficacy was measured in terms of symptom improvement and drug intake reduction. A population of matched patients received drug therapy alone and represented the control group.

## Material and Methods

### Study Design

The study was designed as open-controlled. Two matched groups of patients received either the standard pharmacotherapy or pharmacotherapy plus local IT for three consecutive years. All patients underwent a 1-year run-in period, to assess their baseline conditions. The clinical parameters considered were the symptom score and the drug consumption during the pollen seasons (run in and after 3 years of IT). All patients underwent an assessment of their respiratory function and a methacholine provocation test at baseline and at the end of the study.

### Patients

Fifty-one outcoming patients (25 men and 26 women, mean age 27.4 years, range 15–48) suffering from seasonal rhinoconjunctivitis and/or asthma (mild intermittent or mild persistent) [5] were enrolled. All patients were sensitized to grass pollen only, as confirmed by skin tests (Lofarma SpA, Milan) and RAST (CAP System EIA, Pharmacia Upsala, Sweden) performed with a panel of common allergens (mites, grass, *Parietaria*, olea, compositae, birch, cat and dog dander). Subjects suffering from systemic diseases, major anatomical alterations of the upper airways, receiving chronic corticosteroid or beta-blocking treatments were not admitted, nor were pregnant women. After the run-in, consecutive patients were alternately allocated into two groups

receiving pharmacotherapy only ( $n = 25$ ) or pharmacotherapy plus IT ( $n = 26$ ). Obviously, neither the patient nor the investigators could choose the allocation group. All patients signed an informed consent form.

### Investigational IT and Concomitant Pharmacotherapy

The IT was performed with grass allergoid prepared in monomeric form [6] and incorporated in oral soluble tablets (LAIS, Lofarma SpA, Milan, Italy). The tablets had to be dissolved in the mouth in 1–2 minutes and then swallowed. The vaccine was titrated in Biological Units (Allergen Units, AU) [7] and standardized in potency by RAST-inhibition procedure in comparison to in-house reference. Each preseasonal course, from January to March, involved the administration of a cumulative dosage of about 36,000 AU. All patients were prescribed a standard drug therapy. The prescribed drugs were: oral antihistamines (cetirizine or loratadine 1 tablet 10 mg), topical antihistamine (levocabastine eyedrop bid); nasal flunisolide (50  $\mu$ g bid), inhaled albuterol (1–2 puff 100  $\mu$ g on demand); inhaled fluticasone propionate (250–500  $\mu$ g daily); inhaled sodium cromoglycate (2 mg tid). For severe rhinitis, oral prednisone (1 mg/kg/day for 3–4 days) was also allowed.

### Respiratory Function and Methacholine Test

All patients underwent a respiratory function test (Jaeger, Hochberg, Germany) and a methacholine (MCh) provocation test out of the pollen season (usually in November–January) at run in and after 3 years. A baseline  $FEV_1 \leq 75\%$  of the predicted was required. The methacholine (Lofarma SpA, Milan, Italy) provocations were performed, through a standard procedure [8], using of a Mefar MB3 (Brescia, Italy) dosimeter: each dose output was activated by the patient's inhalatory effort. Increasing doses of MCh (cumulative dose 125–1800  $\mu$ g) were administered at 5-minute intervals. The test was stopped when a 20% (or higher) fall in  $FEV_1$  was achieved and the PD<sub>20</sub> (provocation dose 20%  $FEV_1$ ) was calculated by a proper program. A PD<sub>20</sub> of 1800  $\mu$ g or less was considered indicative of bronchial hyper-reactivity [9].

### Diary Card for Symptoms and Drug Intake

Patients were asked to record on a proper diary card both clinical symptoms and drug intake during the

pollen seasons (April–June) at the run in and after 3 years of treatment. Nasal symptoms (itching, sneeze, rhinorrhea, blockage) and respiratory complaints (dyspnea, cough) were graded as: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. Each dose of albuterol, oral or topical antihistamine, cromolyn, and nasal steroid was scored as 1; each dose of inhaled steroid was scored as 2. A score of 1 was assigned for each milligram of oral prednisone. Finally, each patient receiving the investigational IT was required to fill a proper log for each adverse event possibly related to the intake of the vaccine. A qualitative subjective judgment concerning the clinical efficacy of the local IT was expressed by the patients at the end of the 3rd year.

## Statistical Analysis

Since the clinical scores might be non-normally distributed, the Wilcoxon test for intragroup comparison and the Mann-Whitney *U* test for intergroup comparison were used. Student's *t* test was employed for comparing the results from MCh challenge. *P* values less than 0.05 were considered significant.

## Results

All patients completed the study. The two groups of patients were homogeneous and matched (Table 1). Some of the patients receiving IT reported oral itching or somnolence, but in no case was a dose adjustment or pharmacological treatment required. These effects were mild and self-limiting, and often unrelated to the treatment. The pollen counts during the seasons of interest (kindly provided by Dr. Antonietta Melchiorre, Allergy Service, Desenzano Hospital, Brescia) are plotted in Figure 1: the counts in April–June were higher in the 3rd year than at baseline. Patients receiving IT judged the treatment as

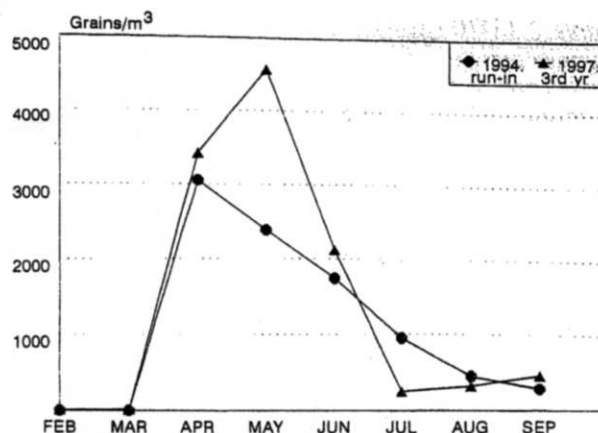


Figure 1. Monthly cumulative pollen counts for grasses in the region of interest at the run-in (1994) and after 3 years of IT (1997)

excellent (15%), good (77%), or of moderate efficacy (8%); no patient judged the treatment unsatisfactory.

## Bronchial Hyperreactivity

Twenty out of the 26 IT-treated patients, and 18 out of the 25 controls, had a MCh bronchial hyperreactivity at enrollment. Since the respiratory function was assessed out of the pollen season, none of them had a  $FEV_1 < 75\%$ . A significant ( $p = .01$ ) increase of PD<sub>20</sub>, and thus a reduction of bronchial hyperreactivity, was observed in the patients receiving IT but not in the control group. The results of the MCh tests are summarized in Table 2.

## Clinical Scores

The scores were considered separately for symptoms and drug intake and for rhinitis and asthma. The two groups were homogeneous at baseline for all these parameters ( $p = NS$ ). A clinical improvement of rhinitis was seen in both groups (Figure 2), but the significance was more relevant in patients receiving IT ( $p = .001$  versus  $p = .01$ ); moreover, a reduction of drug intake was observed only in the IT group ( $p = .001$ ). As far as asthma is concerned, a significant decrease of both symptoms and drug intake ( $p = .001$ ) was seen only in the IT group (Figure 3). A significant intergroup difference ( $p < .05$ ) was present at the 3rd year for all parameters but rhinitis symptoms.

Table 1. Description of the patient population

	IT group	Control group
Number	26	25
Sex (M/F)	13/13	12/13
Mean age (years)	26.4	28.4
Age range (years)	15–45	18–48
Rhinitis only	8	10
Rhinitis+Asthma	18	14
Asthma only	–	1
Bronchial hyperreactivity	20	18
Smokers	0	0

Table 2. PD20 ( $\mu\text{g}$ ) at the MCh test in the two groups at baseline and after 3 years

Patients	IT group		Controls	
	Before	After	Before	After
1	450	750	300	450
2	600	1200	450	600
3	750	900	600	600
4	1200	1800	1200	1200
5	1500	1800	450	450
6	450	900	900	750
7	300	450	900	600
8	450	450	450	1500
9	600	1200	600	1800
10	750	1500	450	600
11	1200	1800	600	750
12	1200	900	750	750
13	1350	1800	1200	1200
14	1500	1800	1500	1800
15	450	750	450	450
16	600	1200	600	750
17	750	1200	1500	1800
18	900	900	1200	1200
19	1200	1500		
20	750	950		
Mean	848	1188	800	958
SD	381	454	368	488
<i>P</i> ( <i>t</i> test)	.01		NS	

## Discussion

Local immunotherapies (intranasal and sublingual/swallow) are presently accepted as viable alternatives to injection IT. This statement is based on the analysis of the literature, which clearly demonstrated the clinical effectiveness of these routes. The IT investigated here was administered as orosoluble tablets and therefore it allowed involvement of both local and systemic immunity, as suggested by the pharmacokinetics study [4]. Since most have studies focused on the effects of IT on rhinitis, we aimed at evaluating also the possible effects on asthma, by means of an objective test. The MCh provocation test was chosen for its simplicity, reproducibility, and safety and because it reflects, to a certain degree, the underlying bronchial inflammation [10]. If an effect of IT on allergic inflammatory phenomena occurs, a reduction of bronchial nonspecific hyperreactivity is also to be expected. In fact, a significant decrease in bronchial nonspecific hyperreactivity was evidenced in patients receiving IT. These observations are consistent with those of Bousquet [11, 12], who demon-

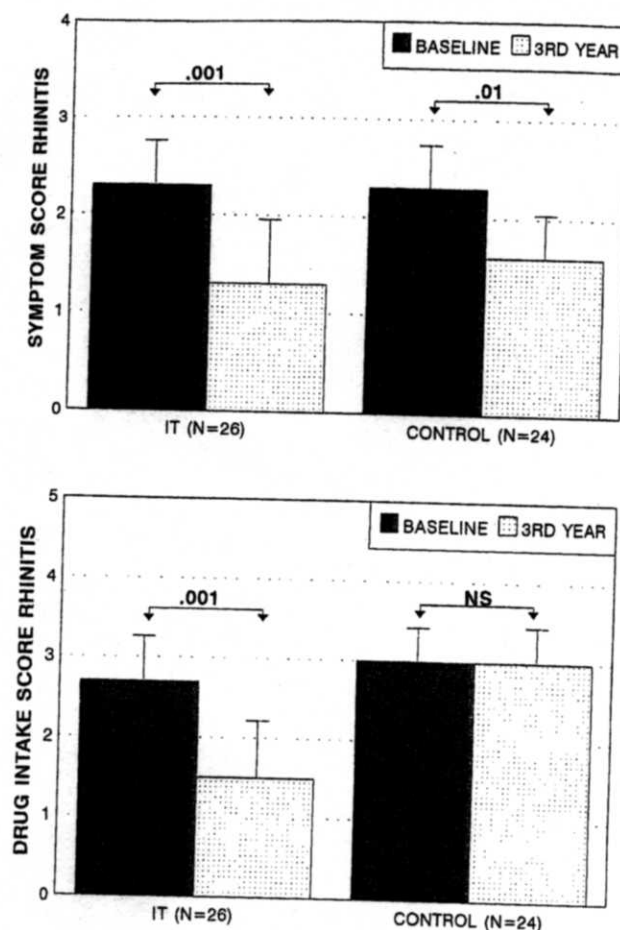


Figure 2. Daily mean ( $\pm$  SD) of symptom score (upper panel) and drug intake score (lower panel) for rhinitis in the two groups at baseline and after the 3rd year of treatment. The *p* values are shown in the graph

strated that IT is capable of improving the pulmonary function of asthmatic subjects, and of Pichler [13], who reported a decrease of nonspecific bronchial hyperreactivity in mite-allergic patients receiving injection IT. The effect on bronchial reactivity was paralleled by the clinical effectiveness of IT: the clinical improvement (as measured by diary card) was highly significant for asthma symptoms and antiasthma medications.

The study was not double blind, but many proofs of the clinical efficacy of sublingual IT are presently available. Moreover, the difficulty of keeping such a number of selected patients double-blind for 4 years has also to be taken in account. In order to correctly evaluate the effects of IT on bronchial hyperreactivity we preferred to have a large number of patients in an open study rather than a small number in a double-blind trial. The measurement of clinical outcomes after 3 years was decided since this is the



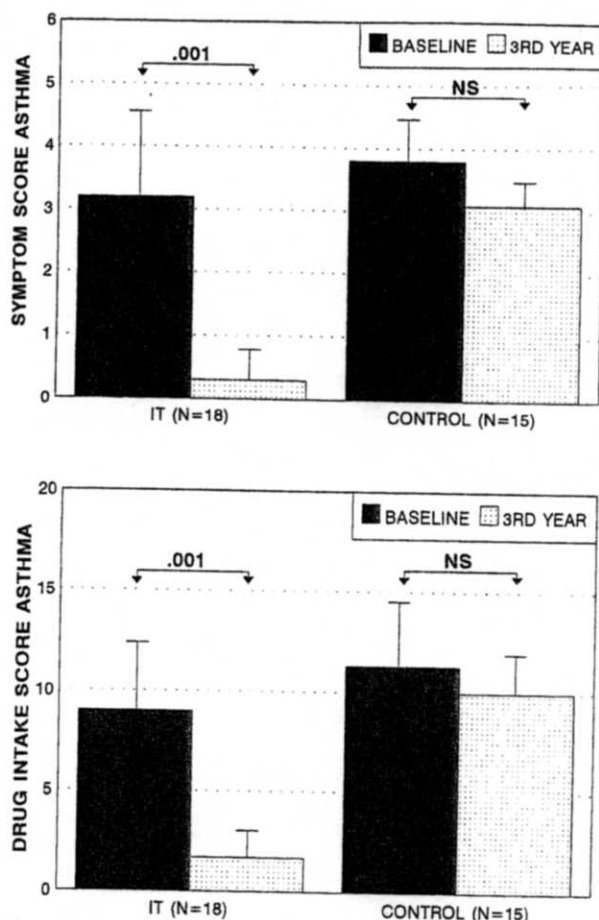


Figure 3. Daily mean ( $\pm$  SD) of symptom score (upper panel) and drug intake score (lower panel) for asthma in the two groups at baseline and after the 3rd year of treatment. The *p* values are shown in the graph

suggested optimal duration for IT to inhalant allergens [2].

In conclusion, the local IT investigated here was able to decrease the nonspecific bronchial hyperreactivity of asthmatic patients: this represented a measurable pathophysiological counterpart of the clinical effects. Finally, the local IT was effective and safe: if administered in association with pharmacotherapy it improved both rhinitis and asthma symptoms and reduced the need for drugs.

## References

1. Passalacqua, G., Canonica, G. W. Alternative routes for allergen specific immunotherapy. A review. *J Invest Allergol Clin Immunol* 1996, 6:81-87.
2. WHO Position Paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. Bousquet, J., Lockey, R. F., Malling H. J. (Eds.), *Allergy* 1998, 54 (Suppl 52).

3. Malling, H. J., Abreu-Nogueira, E., Alvarez Cuesta, E., Bjorksten, B., Bousquet, J., Caillot, D., Canonica, G. W., Passalacqua, G., Saxonis-Papageorgiou, P., Valovirta, E. EAACI Position Paper: Local immunotherapy. *Allergy* 1998, 53:933-944.
4. Bagnasco, M., Mariani, G., Passalacqua, G., Motta, C., Bartolomei, M., Falagiani, P., Mistrello, G., Canonica, G. W. Absorption and distribution kinetics of the major *Parietaria judaica* allergen (Par j 1) administered by noninjectable routes to healthy human beings. *J Allergy Clin Immunol* 1997, 100:121-129.
5. Global initiative for asthma. *NHLBI/WHO Workshop report*. NIH publication 95-3659; jan 1995.
6. Mistrello, G., Brenna, O., Roncarolo, D., Zanoni, D., Gentili, M., Falagiani, P. Monomeric chemically modified allergens. Immunologic and physicochemical characterization. *Allergy* 1996, 51:8-1.
7. Giannarini, L., Maggi, E. Decrease of allergen-specific T cell response induced by local nasal immunotherapy. *Clin Exp Allergy* 1998, 28:404-412.
8. Eiser, N. M., Kerrebijn, K. F., Quanjer, P. H. Guidelines for standardization of bronchial challenges with non-specific bronchoconstricting agents. *Bull Eur Physio-path Resp* 1983, 19:495-500.
9. Hargreave, F. E., Ryan, G., Thomson, N. C., O'Byrne, P. M., Latimer, K., Juniper, E. F., Dolovich, J. Bronchial responsiveness to histamine and methacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol* 1981, 68:347-355.
10. Bradley, B. L., Azzawi, M., Jacobson, M., Assoufi, B., Collins, J. V., Irani, A., Schwartz, L. B., Durham, S. R., Jeffery, P. K., Kay, A. B. Eosinophils, T lymphocytes, mast cells, neutrophils and macrophages in bronchial biopsy specimen from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1991, 88:661-674.
11. Clavel, R., Bousquet, J., André, C. Clinical efficacy of sublingual swallow immunotherapy: a double blind placebo controlled trial of a standardized five grass pollen extract in rhinitis. *Allergy* 1998, 53:493-498.
12. Bousquet, J., Scheinmann, P., Guinépain, M. T., Perrin-Fayolle, M., Sauvaget, J., Tonnel, A. B., Pauli, G., Caillaud, D., Dubost, R., Leynardier, F., Vervloet, D., Herman, D., Galvain, S., André, C. Sublingual swallow IT in patients with asthma due to house dust mites: a double blind placebo controlled study. *Allergy* 1999, 54:249-260.
13. Pichler, C. E., Marquardsen, A., Sparnholt, S., Lowenstein, H., Bircher, A., Bischof, H., Pichler, W. J. Specific immunotherapy with dermatophagoides pteronyssinus and farinae results in decreased bronchial hyperreactivity. *Allergy* 1997, 52:274-283.

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