

EVALUATION OF THE ANTIINFLAMMATORY AND CLINICAL EFFECTS OF SUBLINGUAL IMMUNOTHERAPY WITH CARBAMYLATED ALLERGOID IN ALLERGIC ASTHMA WITH OR WITHOUT RHINITIS. A 12-MONTH PERSPECTIVE RANDOMIZED, CONTROLLED, TRIAL

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SUMMARY:

■ **Background:** The efficacy and safety of sublingual immunotherapy with carbamylated allergoid (allergoid SLIT) is well recognised. Yet, few data concerning its antiinflammatory effects on the respiratory airways are so far available. Thus we decided to evaluate whether it can reduce the allergic inflammation and improve the clinical symptoms in comparison to pharmacotherapy.

■ **Methods:** The study was perspective, controlled and randomised. It was conducted on 56 patients allergic to House Dust Mite with ($n=36$) or without *Parietaria*. Thirty-three of them were allocated to SLIT (22 M, 11 F mean age 15 years) and 22 (13 M, 10 F, mean age 21 years) to pharmacotherapy. They were followed-up for 1 year. Symptoms and drugs consumption were assessed by monthly diary cards. Bronchial reactivity was investigated at baseline and after a 12-month treatment, through a methacholine (MCh) test. An evaluation of the nasal eosinophils was also performed at the same times.

■ **Results:** There was a greater reduction of the mean symptom score ($p<0.01$) and drug consumption ($p<0.001$) in the SLIT than in the control group. MCh PD₂₀ increased only in the SLIT group ($p<0.0005$). The reduction of nasal eosinophils was statistically greater ($p<0.05$) only in the SLIT group.

■ **Conclusions:** A 1-year SLIT reduces the allergic symptoms and the respiratory airways inflammation more than pharmacotherapy.

Key-words: Asthma - Antiinflammatory effects - Asthma - Carbamylated allergoid - Sublingual immunotherapy - Rhinitis.

INTRODUCTION

Allergen-specific immunotherapy is of relevant importance in the prevention and treatment of respiratory allergy and its clinical value is nowadays well recognised (1-3). In the last twenty years new routes of administration have been investigated and developed. Among these, the sublingual route (SLIT) appeared the most promising alternative to the traditional IT. At present the efficacy and safety of sublingual allergen-specific immunotherapy (SLIT), in the management of respiratory allergy, are fully established [4-9]. Yet there are quite few data concerning its effects on inflammation of the upper and

lower airways (10-14). Thus we decided to evaluate whether SLIT can improve the clinical situation and, in addition to that, reduce the allergic inflammation in comparison to pharmacotherapy (controls)

MATERIAL AND METHODS

Study design

The study was perspective open-controlled, randomised in a 3:2 ratio, two parallel groups receiving either SLIT or the standard pharmacotherapy for mild persistent asthma (15). Both groups were allowed to receive rescue medication, on demand for very short periods (no more than some days). No run-in period was scheduled. All patients were evaluated at entry to assess their baseline conditions. The clinical parameters considered were the symptom scores and the drug consumption during the whole study duration

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(12 months). In addition, all patients underwent an assessment of their bronchial hyperreactivity (BHR) through a methacholine (MCh) test at baseline and at the end of the study as well as an evaluation of their nasal inflammation by means of a nasal smear for eosinophil count.

Patients

Fifty-six outcoming patients suffering from mild persistent asthma (15) with or without intermittent-moderate rhinitis were enrolled. Thirty-three patients were allocated to SLIT (22 M, 11 F mean age 15 ± 9 years) and 23 (13 M, 10 F, mean age 21 ± 15 years) to pharmacotherapy. All the 56 patients were sensitised to house dust mite and 36 also to *Parietaria* as confirmed by a positive (> 3 mm) skin prick test response (Lofarma S.p.A., Milan) and positive CAP-RAST assay result (class II or greater) (CAP System EIA, Pharmacia Upsala, Sweden). Subject suffering from systemic or immunological diseases, major anatomical alterations of the upper airways, renal insufficiency, CHD, neurologic or psychiatric diseases, receiving chronic corticosteroid or beta-blocking treatments were not admitted nor were pregnant women. Finally, was excluded from the study patients with no BHR (defined as MCh PD₂₀ of 1800 μ g or less at baseline out of pollen season) and/or no nasal inflammation (i.e. with an eosinophil grade $< 2+$ at the nasal smear). All patients signed an informed consent before entering the study.

Investigational SLIT and Concomitant Pharmacotherapy

SLIT is a monomeric carbamylated allergoid (Lais®, Lofarma S.p.A. Milan, Italy) biologically standardised [16] in allergenic units (AU) and prepared as soluble tablets (allergoid SLIT). The tablets had to be taken in the morning on an empty stomach and kept under the tongue for 1-2 minutes until dissolution before swallowing. During a quite rapid build-up phase of about 16 days, tablets with increasing dosages (25, 100, 300 and 1,000 AU) were used, in order to gradually achieve the maximum dose of 1,000 AU. Subsequently, that maintenance dosage of 1,000 AU was administered two times a week for 1 year continuously. Concerning the content of major allergen, it is not reported on the product label because the chemical modification of the allergen does not allow its titration in micrograms. Enrolled patients were randomly allocated to allergoid SLIT or pharmacotherapy according to a computer-generated list with an active-controlled ratio of 3:2.

The rescue medication as needed, for symptoms control, was the following: cetirizine tables (10 mg once daily), inhaled salbutamol (100 μ g 2 puff), intra-nasal fluticasone (50 μ g 1 spray per nostril). In case of severe symptoms, unresponsive to the standard treatment, a short course of systemic steroid was allowed (prednisone 50 mg daily for 3 days).

Clinical evaluation

Patients were required to record daily on a specific form the presence and severity of symptoms and the amount of medication used. The diary had to be filled in during the whole study year, being house dust mite (and *Parietaria*) perennial allergens. The following symptoms were considered: nasal itching, obstruction, rhinorrhea, sneezing, ocular itching and or lacrimation, cough and shortness of breath. A score ranging from 0 (absent) to 3 (severe) was attributed daily to each of the mentioned symptoms. A mean daily score was then calculated for the whole 12-month study period. The rescue drug intake was scored 1 point for each actuation of salbutamol, 2 points for each dose of antihistamine, nasal or inhaled steroid, 3 points for each dose of systemic steroid, and a cumulative yearly drug intake was obtained. All patients were also required to record on a separate diary any untoward effect. As far as the allergoid SLIT is concerned, adverse events (AE) were subdivided into local AE (oral itching, swelling of tongue) and systemic (asthma, rhinitis, urticaria, abdominal pain/diarrhea, anaphylaxis).

Methacholine challenge

MCh bronchial provocation test was performed using an ampoule-dosimeter (Mefar Elettromedicali, Brescia, Italy). An inspiratory effort for 0.5 s activated a solenoid valve, delivering 5 mL of solution. Lyophilized MCh chloride (Lofarma S.p.A, Milan, Italy) was reconstituted, to obtain 0.2% and 1.0% concentrations. After saline control, MCh was administered in double increasing amounts: each subject inhaled 1 and 2 breaths of 0.2% MCh solution (each breath corresponding to 10 mg of MCh), followed by 1, 2, 4, 8, 16, and again 16 breaths of 1.0% solution (each corresponding to 50 mg of MCh). FEV₁ was recorded about 2 minutes after each MCh dose. All bronchial challenge were performed at the same hour of the day under the same environmental conditions. The MCh test was done out of the pollen season. The cumulative administered dose of MCh causing a reduction of 20% of the baseline FEV₁ (PD₂₀) was computed by interpolating the MCh cumulative doses immediately preceding and following the 20% fall of FEV₁.

■ **Degree of BHR:** Three arbitrary classes of BHR were considered in our study: very mild = $PD_{20} > 800 \mu\text{g/ml}$, mild = PD_{20} ranging from 400 to 800 $\mu\text{g/ml}$ and moderate = $PD_{20} < 400 \mu\text{g/ml}$.

■ **Nasal eosinophils:** The cytological drawings have been performed by the scraping technique, smearing 2-3 times a rhinological probe (Rino-Probe) on the mucosal surface of the central zone of the inferior turbinate. The material so collected has been transferred on microscope slides and laid as monolayer. The slides have been fixed with 95° ethanol and stained by May-Grunwald-Giemsa method, which is able to stain all the cellular elements present both in normal and inflammatory conditions. The reading of slides by optical microscope has been made on different fields on the whole slide's surface in order to observe all the cellular elements. The examination has been made by a blinded operator (not involved in the clinical study) that counted the various inflammatory cells (eosinophils, neutrophils, lymphocytes and mast cells). Yet, to evaluate the severity of the allergic inflammation only the eosinophils were considered.

To determine this one we used the so called "inflammation degree", that is a numeric index ranging from 0 (no elements) to 4+ (big cellular mass covering entirely the microscope field) that correlates quite well with the inflammatory conditions of the tissue [17]. A baseline cytological examination at enrolment of patients (March 2004) and a second examination after one year (March 2005) have been performed.

■ **Statistical analysis:** To determine whether the values of a particular variable differ between the two

populations (active versus controls) at baseline, the Mann-Whitney U test for intergroup comparison was used. The Wilcoxon test for intragroup comparison was employed for comparing the results from symptoms score, VAS, MCh challenge and nasal eosinophils, before and after treatment. P values less than 0.05 were considered significant.

RESULTS

Patients

All the 56 enrolled patients completed the study whose design is shown in figure 1. No patient dropped out during the study. The two groups of patients were homogeneous at baseline for demographic and clinical characteristics (table 1).

■ **Clinical parameters:** A significantly greater reduction in the mean daily clinical score (all symptoms, $p < 0.01$) and drug consumption (all drugs, $p < 0.001$) was observed in the SLIT than in the control group during the whole treatment period (figure 4).

■ **Bronchial hyperreactivity:** A significant increase of MCh PD_{20} ($p < 0.0005$) was observed, after one year, only in the group of patients treated with pharmacotherapy plus SLIT (table 2). This datum did not change even when we considered the individual trend of each patient (figure 2). On the other hand, dividing the patients according to the three arbitrary classes of BHR described above, we observed an improvement of the BHR that was proportionally more pronounced in the patients with the lower PD_{20} values both in the active (Figure 3a) and in the control group (figure 3b).

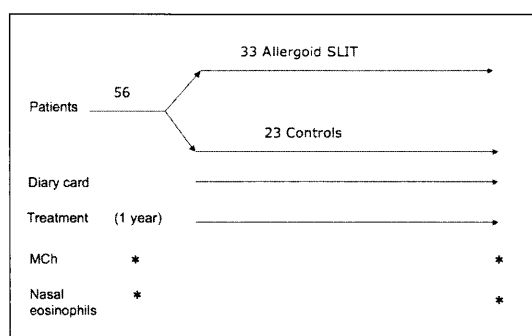


Figure 1: Study design.

	Allergoid SLIT	Controls	P Value
Patients	33	23	NS
Mean age (years)	15.4	21.8	NS
Range	8 - 44	7 - 68	
Sex (M/F)	22/11	13/10	NS
Methacholine PD_{20} (μg)	626.4 ± 526.19	616.1 ± 578.08	NS
Nasal eosinophils (grading)	2.0 ± 1.14	2.1 ± 0.52	NS

Table 1: Demographic data at baseline.

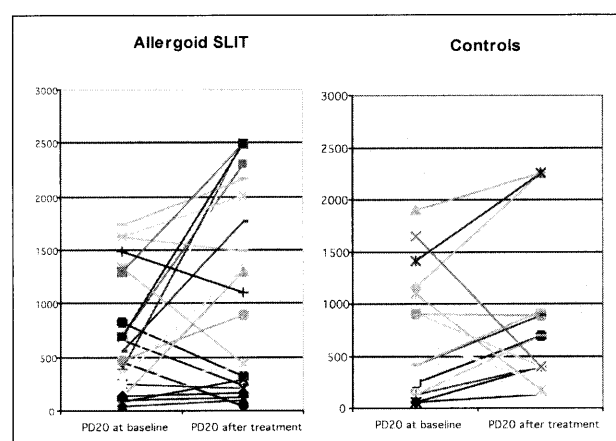


Figure 2: Methacholine PD_{20} individual values (μg) at baseline and after treatment in the two groups.

■ **Nasal eosinophils:** Nasal eosinophils decreased significantly in the two groups but the reduction was greater both from a clinically and statistically point of view in the allergoid SLIT group (table 3).

Adverse events

Neither local nor systemic relevant adverse events were observed during the study in any of the two groups.

DISCUSSION

At the moment there are many clinical controlled trials indicating the clinical efficacy, the safety and the tolerability of SLIT and, in particular, as far as the last two points are concerned, of the allergoid SLIT (5, 6, 8, 14). In addition, SLIT is capable of improving the patients' QoL [18] and to prevent the development of asthma in children with allergic rhinoconjunctivitis

(19). Nevertheless, some aspects of SLIT, as its effects on the inflammation of the lower and upper respiratory airways, though recently addressed by some authors [10-14], could be further elucidated.

In our study, differently from what done by the previous authors, we decided to investigate whether the addition to pharmacotherapy, of a SLIT starting with a quite rapid build-up phase reaching, during the last three days, a rather high daily dosage (2000 AU) and continuing with a maintenance phase of 2000 AU weekly, would allow to decrease the above mentioned inflammation in a time shorter than 2-3 years (10, 12, 14). As control group we decided to choose the pharmacological therapy usually employed in asthmatic and rhinitic patients that is already an effective anti-inflammatory treatment so as to better demonstrate any eventual "ad-on effect" of SLIT. We decided not to use a double-blind placebo-controlled design because nowadays, in Italy, it is not well accepted by ethical committees, doctors and patients as well.

In order to make clearer the study results we chose to utilise only three simple measurement parameters: two objectives (the changes in the BHR, evaluated with the MCh challenge and the nasal eosinophils measured by means of the "inflammation degree" and one subjective (the clinical improvement assessed by the symptoms and the drug consumption reduction). As previously specified, this subjective parameter refers to the whole study period (i.e. the diary card were filled in during all the 12 months of the study) being house dust mite a perennial allergens and *Parietaria* a pollen with particularly prolonged pollination in the south of Italy (20).

It was observed a significant improvement of both the objective parameters after one year of treatment. On the contrary, there were neither statistical nor clinical

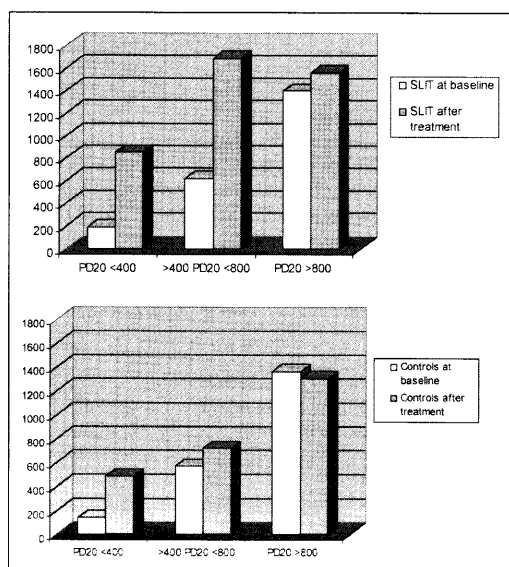


Figure 3: Methacholine PD₂₀ mean values at baseline and after treatment in the two groups according to the baseline methacholine PD₂₀ value indicating three arbitrary classes of BHR: very mild (PD₂₀>800 µg/ml), mild (PD₂₀ ranging from 400 to 800 µg/ml) and moderate (PD₂₀<400 µg/ml).

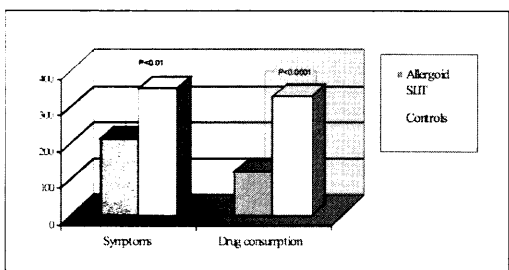


Figure 4: Daily mean symptom score and drug consumption in the two groups during the study.

	Allergoid SLIT	Controls
Methacholine PD ₂₀ (µg) at baseline	626.4 ± 526.19	616.1 ± 578.08
Methacholine PD ₂₀ (µg) after treatment	1277.7 ± 963.51	860.3 ± 732.39
P Value	0.001	0.08

Table 2: Methacholine PD mean values (µg) at baseline and after treatment in the two groups.

	Allergoid SLIT	Controls
Nasal eosinophils at baseline	2.0 ± 1.14	2.1 ± 0.29
Nasal eosinophil after treatment	1.0 ± 1.03	1.4 ± 0.52
P Value	0.00001	0.01

Table 3: Nasal eosinophils in the nasal smear at baseline and after treatment in the two groups.

differences between patients allergic to house dust mites and those allergic to *Parietaria*. This datum is not surprising. Being both the allergens perennial in the south of Italy [20], it can be expected that they may behave similarly. Furthermore the number of patients in each group is too small in order to appreciate any difference.

As far as the BHR is concerned, this improvement was much greater, as expected, in the patients with a more severe disease at baseline, expressed by lower PD20 values. This phenomenon was observed in both the groups but was both clinically and a statistically more evident in the SLIT group than in the controls. Also this datum is not new at all, being known that the difference between an active and a control group is greater the more the disease is severe.

A greater reduction of the nasal eosinophils was seen in the active group but, in this case, a significant statistical and clinical difference was present also in the control group, even if smaller.

The effects on bronchial and nasal inflammation were paralleled by the data concerning the subjective parameters: the clinical improvement observed with the allergoid SLIT was in fact highly relevant, both for symptoms and drug consumption reduction, in comparison to the pharmacotherapy alone.

Our observations are consistent with those of Lombardi (14), Marogna (10, 13) and Pajno (12) who reported that SLIT is capable of decreasing nonspecific BHR and nasal eosinophils in allergic patients.

In addition to that, our data show that, in asthmatic patients with or without rhinitis due to perennial allergens (house dust mite with or without *Parietaria* in this case), a perennial SLIT course lasting only 12-month allows to obtain similar results without any remarkable adverse event.

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