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Original article

Low-dose local nasal immunotherapy in children with perennial allergic rhinitis due to *Dermatophagoides*

Background: Allergen specific immunotherapy was known to be useful in the treatment of respiratory allergic disease. Local nasal immunotherapy (LNIT) offers advantages such as a good efficacy/safety ratio and a more convenient allergen delivery. The aim of this study was to assess the safety and clinical efficacy of a modified scheduling of LNIT in 32 children with allergic rhinitis due to *Dermatophagoides*.
Methods: A multicentre, randomized, double-blind placebo controlled study carried out for two years, with a modified schedule of LNIT treatment: a build-up phase at increasing dosages from 2.5 AU to 80 AU and a maintenance period at low dosage (80 AU) once a week. Symptom and medication scores, threshold dose with specific nasal provocation test (NPT) and immunological parameters (IgE and IgG_4) were evaluated.
Results: No important local or systemic side-effects were observed in children who completed the study. Compared to placebo, the active treatment group showed significant improvement in rhinitis symptoms and a reduction of drug consumption after 18 months of LNIT. These results were confirmed by a significant reduction of allergen specific nasal reactivity. Serum and nasal specific IgE and IgG_4 did not show any difference in the two groups.
Conclusions: The safety and clinical efficacy of low-dose LNIT suggests that this therapy may be useful in the treatment of allergic rhinitis disease in children.

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Key words: allergic rhinitis; children; *Dermatophagoides*; local nasal immunotherapy; nasal provocation test.

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Many controlled studies confirm the clinical efficacy of allergen specific immunotherapy and its relative safety if properly administered (1). Local immunotherapy for respiratory allergy was conceived firstly by Dunbar in 1913 adopting the nasal route (2), and secondly by Herxheimer in 1951 adopting the bronchial route (3). Successive studies in experimental animals have showed that allergens administered onto the nasal mucosa are able to elicit a significant systemic antibody response towards the allergenic components of pollen extract (4). Experimental studies performed in atopic and non-atopic subjects (5) and in rabbits (6) showed the ability of nasal mucosa to absorb allergenic molecules, which can be detected biologically active with their antigenic properties unmodified in the bloodstream. Controlled clinical trials have been performed with allergens in liquid form, natural and polymerized allergoids, and have showed the clinical efficacy of local nasal immunotherapy (LNIT) in grass and ragweed allergic rhinitis (7–11). Problems with the stability of liquid allergens led to the development of allergen in lyophilized macronized powder. This product has been submitted to double-blind placebo-controlled studies of *Parietaria*, *Dermatophagoides*, grass and birch pollen-induced rhinitis, and this route of administration of the allergen specific immunotherapy has been shown to have clinical efficacy and safety (12–20). LNIT offers some advantages over parenteral immunotherapy, with no risk of life-threatening reactions, and more convenient allergen delivery. Furthermore, the effect can be monitored directly on the target organ, evaluating the nasal reactivity by a nasal allergen specific provocation test (NPT). This test could be used to establish the dose to be delivered, just below the provocative threshold, i.e. to further improve the efficacy/safety ratio, mainly in special populations as children. In this trial we assessed the safety and clinical efficacy of this innovative approach with a low-dose LNIT in children with allergic perennial rhinitis due to *Dermatophagoides*.

Material and methods

Study design

A multicentre, randomized, double-blind placebo controlled trial was carried out in a group of allergic children with perennial rhinitis

due to *Dermatophagoides*. The aim of this clinical trial was to evaluate the efficacy and safety of LNIT according to a partially modified schedule of treatment. The study started in September 1994 and lasted until March 1996. The trial was performed in three university centres: the Pediatric Clinics of Perugia, Modena, and Florence and Parma and was approved by the Local Ethical Committees. Written informed consent was signed by parents for each patient.

Patients (Table 1)

Thirty-two outpatients were enrolled (14 female and 18 male) aged between 4 and 14 years (9.3 ± 2.9), with perennial allergic rhinitis to *Dermatophagoides*. The inclusion criteria were as follows.

1. Perennial rhinitis for the previous two years.
2. A history characterized by early morning and night symptoms, symptoms indoors and particularly after exposure to house dust.
3. Positive skin prick tests ($>/=2$ plus) and serum radio-allergosorbent test (RAST) to *Dermatophagoides* ($>/=$class 2).
4. Positive nasal provocation test to mites.
5. Negative skin prick test and serum RAST for other perennial aeroallergens.

Exclusion criteria were: bronchial asthma, seasonal rhinitis due to pollens, nasal polyposis or other mechanical obstruction, severe systemic disease, systemic or local steroid treatment or specific immunotherapy during the previous three years. Other exclusion criteria were the contraindications for immunotherapy of the European Academy of Allergy and Clinical Immunology (EAACI) (21).

Patients were assigned randomly to the treatment according to a computer-generated list.

Allergen specific nasal provocation test

The specific NPT, carried out to assess nasal reactivity, involved challenge with increasing concentrations of *Dermatophagoides* allergenic extract in powder form (2.5, 5, 10, 20, 40, 60, 80, 120, 160 AU; Allerkin Test®, Lofarma SpA, Milan, Italy) every 20 min until the threshold dose was reached.

A control capsule of lactose was sprayed into one nostril before starting the challenge.

Threshold doses of allergen were considered those amounts required to elicit at least two of four nasal symptoms: itching, sneezing, discharge and obstruction. The NPT was done before starting treatment (October 1994), after 5 months (March 1995) and at the end of the immunotherapy period (March 1996).

All patients were asked to continue their normal house cleaning activities in order to maintain the same level of house dust mite exposure.

Immunological assays

Serum samples and nasal secretions were collected and specific IgE and IgG_4 to *Dermatophagoides* were assayed according to the CAP-System FEIA method (Pharmacia, Uppsala, Sweden). Specific IgE and IgG_4 in nasal secretions were evaluated according to a previously described technique (22). Briefly, the same substrate used for the serum assay (the sponge removed from a CAP plastic container) was applied within a permeable membrane to the nasal mucosa and allowed to incubate for 10 min. The cellulose sponge was then removed, placed in a nylon container and stored in 0.9% NaCl, NaN_3 (sodium azide) 0.02% solution at $-20^{\circ}C$ until processing. After washing with 0.9% NaCl and Tween 20 1% solution, the sponge was replaced in the CAP plastic container and the specific IgE, IgG_4 levels were assayed with the same procedure used for the serum.

Allergen preparation

The active product (Allerkin®; Lofarma) was in powder form and consisted of a mixture of mite extracts (50% *Dermatophagoides pteronyssinus* and 50% *Dermatophagoides farinae*), incorporated into inert lactose. Increasing dosages (2.5, 5, 10, 20, 40, 60, 80 AU) were prepared and enclosed in hard gelatine capsules. Placebo was lactose powder indistinguishable from the active treatment. The product was titrated in allergenic units (AU) and standardized by RAST inhibition in comparison with an in-house reference preparation. One AU is 1/40 of the mean provocative threshold in the nasal challenge in allergic volunteers.

Treatment schedule

The treatment was given according to a schedule at increasing dosages from 2.5 AU to 80 AU.

The LNIT was self-administered using a specific device on alternate days, three times per week, into alternate nostrils. Each dose was administered six times. After this induction period each patient followed a period of maintenance at 80 AU once weekly, until reaching a cumulative dosage of 5780 AU.

The maintenance dose of allergen (80 AU) was determined on the basis of previous observations to assess the better clinical/safety ratio in a pediatric population.

Clinical evaluation

All patients were enrolled in the study after a wash-out period of one month.

The main clinical endpoints were evaluated during a window period of 5 months (November–March), corresponding to the maximal exposure to the mite allergens in our country, for two consecutive years.

Efficacy criteria were: physician's clinical evaluation at monthly visits; patients' diary-cards recording symptoms and medication scores during the study; and measurement of the threshold dose with allergen specific NPT.

The patients were examined alternately by two physicians in order to assure a thorough blinding procedure.

The monthly symptom scores (rhinitis, conjunctivitis, nasal discharge, cough and breathlessness) for each patient were obtained from symptoms recorded daily in the parents' diary-cards. Symptoms were rated according to the following scale: 0 = no symptoms; 1 = mild symptoms; 2 = moderate symptoms; 3 = serious symptoms. For each patient the total of rescue medications taken daily (systemic antihistamines, nasal chromoglycate, ocular chromoglycate, beta-2-agonists) was recorded. The information entered on the diary-cards was evaluated using the following scale: 1 point for each application of nasal and/or ocular chromoglycate drops; 2 points for every puff of beta-2-agonist; 3 points for every antihistamine taken. Symptom and medication scores were evaluated as mean values for the two periods of maximal exposure to mites, from November to March.

Tolerability was evaluated in terms of number of side-effects experienced, throughout the whole study period, following administration of each capsule. For each patient the type of symptom, the interval from the dose, the duration of the reaction, the dosage provoking the reaction and the concomitant presence of any other events were recorded in a monthly diary-card. Compliance with treatment was evaluated by counting the remaining capsules at the monthly visit. Patients were defined as compliant if the minimum cumulative dosage of 5000 AU was reached and then evaluated for clinical efficacy.

Immunological parameters such as measurement of specific IgE and IgG_4 in blood and nasal secretions and NPT were assessed before the treatment (October 1994), after 5 months (March 1995) and at the end of the immunotherapy (March 1996).

Table 1 Demographic data

	Active	Placebo
Number of patients	16	16
Mean age (years ± SD)	8.7 ± 2.8	9.7 ± 3.0
Age range	5–14	4–14
Sex (M/F)	10/6	7/9
Mean duration rhinitis (months)	20	18
SPT	>/=2 plus	>/=2 plus
Serum RAST (KU/L)	8.3 ± 6.8	9.1 ± 6.2
NPT threshold dose (AU)	59.2 ± 25.7	53.7 ± 22.7

Statistical analysis

Symptoms and drug intake data were statistically analysed by nonparametric tests. The Wilcoxon test was used for intragroup analysis and the Mann–Whitney *U*-test for intergroup analysis. The same tests were done for NPT results and for specific IgE and IgG_4 levels in serum and nasal secretion.

Results

Twenty-six out of 32 patients (12 active LNIT, 14 placebo) completed the study (14 F/12 M; mean age 9.2 ± 3.1) and reached the minimum cumulative dosage required of 5000 AU (Fig. 1). Four patients in the active group and two in the placebo group dropped out during the induction phase (three in the active group and one in the placebo group because of a rhinitis exacerbation, and one patient in each group for reasons not related to the treatment) before reaching the required cumulative dosage. The two groups were comparable in terms of the main demographic data (Table 1).

There were no relevant local or systemic reactions during the treatment.

Clinical efficacy was evaluated by comparing the mean monthly symptom and medication scores in the active and placebo groups during the first and the second evaluation periods (Figs 2 and 3).

In the first period no differences were observed in either the parameters examined, but in the second period (from November 1995 until March 1996) the active group had significantly fewer nasal symptoms than the placebo group ($P<0.01$) (mean ± standard deviation: active = 25.4 ± 7.33; placebo = 47.7 ± 6.64) (Fig. 2). Over the same period the medication score was also significantly lower ($P<0.01$) in the active group compared to the placebo group (mean ± standard deviation: active = 1.7 ± 0.68, placebo = 4.2 ± 1.5) (Fig. 3).

At the beginning of the trial specific NPT threshold dose was 59.2 ± 25.7 AU (mean ± standard deviation) in the active group, and 53.7 ± 22.7 AU in the placebo group (P = NS) (Fig. 4). After 6 months (March 1995) the specific NPT threshold dose in the active group was 80 ± 30.7 AU and in the placebo group was 67.9 ± 20.8 AU, and at the end of the trial (March 1996), it was 120 ± 41.8 AU and 60.9 ± 40.9 AU, respectively. There was an increase in only the active group, with a significant difference ($P<0.01$) when values at the beginning and at the end of the study were compared. Furthermore, at the end of the trial the difference between the active and placebo groups was significant ($P<0.01$).

Specific IgE, IgG_4 levels in serum samples and nasal secretions were not different in the active or placebo groups, as shown in Table 2.

Discussion

Preliminary data suggest that in children immunotherapy may impede the progression from allergic rhinoconjunctivitis to asthma (23, 24). In asthmatic children allergic to mites, immunotherapy prevented new sensitization during a three-year follow-up (25), suggesting it has potential for the prevention of allergic respiratory disease (26). LNIT has some potential advantages over traditional immunotherapy, mainly the lack of life-threatening reactions and the more convenient allergen delivery. To assess clinical efficacy of LNIT, this study investigated the safety and efficacy of a modified scheduling of LNIT in a selected pediatric population. Previous studies of intranasal immunotherapy using aqueous extract have shown that symptoms of rhinitis arise with high doses (27, 28) whereas low

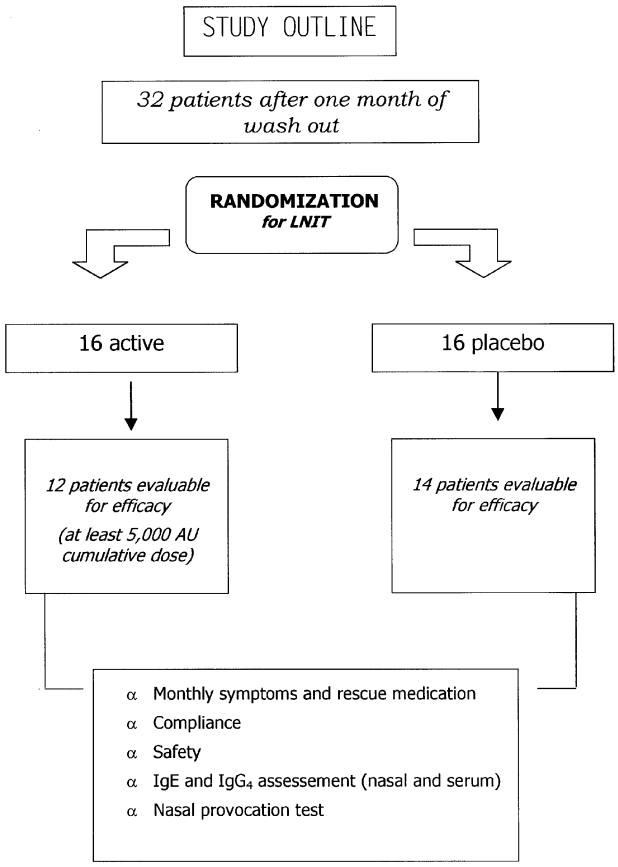

Figure 1 Outline of the LNIT study

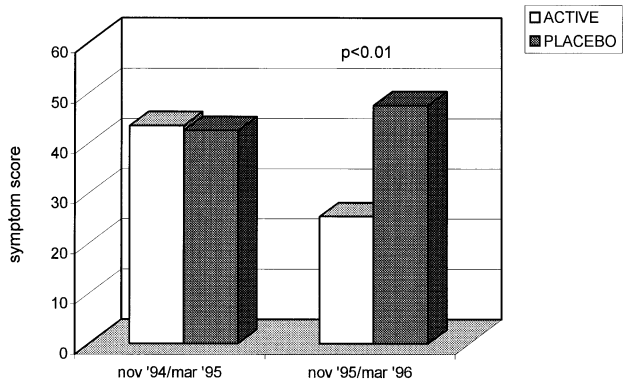

Figure 2 Mean values of symptom scores in the two periods of maximal exposure. A significant difference ($P<0.01$) between active and placebo group was evident in the second period of the trial

doses are well tolerated, but partially lack clinical efficacy (29). With the low-dose LNIT there was significant reduction in the nasal symptom scores after 12 months, comparable to the results of controlled studies with the same treatment at higher doses (13, 17).

Only a few studies have focused on perennial allergic rhinitis treatment with LNIT. In adults allergic to *Dermatophagoides*, Andri et al. reported clinical improvement after six months of therapy (13) and the data have been confirmed by Palma Carlos et al. (30). In children, as showed in our study, symptoms of rhinitis were significantly reduced after 12 months until the end of therapy. This difference may be due to the low dose regimen we employed.

Like symptom scores, medication scores showed a significant decrease after 12 months in the treated group, with a significant difference from placebo. Nasal reactivity to specific allergen provocation was significantly reduced at the end of the treatment.

In this study we observed some drop-outs during the induction phase mainly related to rhinitis exacerbation, possibly due to a local side-effect of immunotherapy.

In a recent paper, Pocobelli et al. reported a

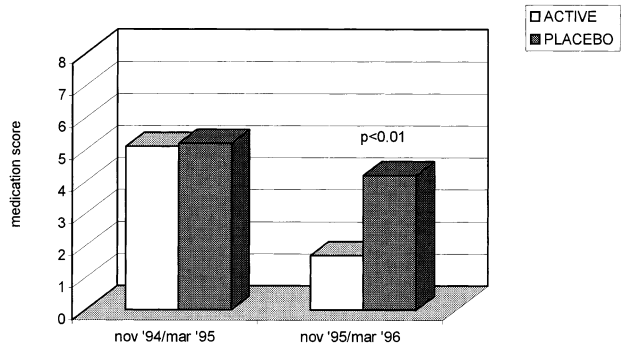

Figure 3 Mean values of medication scores in the two periods of maximal exposure. A significant difference ($P<0.01$) between active and placebo group was evident in the second period of the trial

Table 2 Specific IgE and IgG_4 (mean values ± SD expressed in kU/l)

	Serum		Nasal secretion	
	Active	Placebo	Active	Placebo
IgE-1	8.3 ± 6.8	9.1 ± 6.2	1.3 ± 2.2	1.2 ± 1.3
IgE-2	7.8 ± 6.5	8.7 ± 5.7	0.7 ± 0.7	1.0 ± 1.3
IgE-3	8.0 ± 6.7	8.7 ± 5.3	0.9 ± 1.1	1.5 ± 2.4
IgG_4-1	0.9 ± 0.5	1.3 ± 1.5	1.0 ± 1.3	0.7 ± 0.5
IgG_4-2	1.7 ± 1.8	1.0 ± 0.9	0.8 ± 0.7	0.7 ± 0.4
IgG_4-3	2.0 ± 2.3	1.0 ± 0.8	1.0 ± 1.3	0.7 ± 0.5

1 = October 1994; 2 = March 1995; 3 = March 1996

significant clinical and safety improvement using a constant low dosage scheduling LNIT (40 AU) in adults with grass rhinitis (31). This innovative approach should also be evaluated in children for a further improvement of the tolerability profile during the induction phase.

Some authors reported increases in serum and nasal specific IgG, IgA, and IgE (9, 32) but others found no changes in these immunological parameters (13). In our study we found no significant changes in serum and nasal specific IgE and IgG_4 confirming that clinical benefit is not related to IgE and IgG_4 response. Studies in rodents indicate that repeated inhalation of allergens may be responsible for a switch from TH_2 cells to TH_1 (33). Clinical studies have recently indicated that LNIT may reduce Inter Cellular Adhesion Molecule-1 (ICAM-1) expression on epithelial cells (20), and that both subcutaneous and local nasal immunotherapy may induce a decrease of allergen-specific T cell response (34).

In our study we obtained a significant clinical response with a low-dose scheduling LNIT in children with perennial allergic rhinitis, with good compliance and no relevant side-effects. This preliminary finding suggests LNIT at low dosage may be useful in the treatment of pediatric nasal allergy. Long-term efficacy and the preventive capacity of LNIT have not yet been

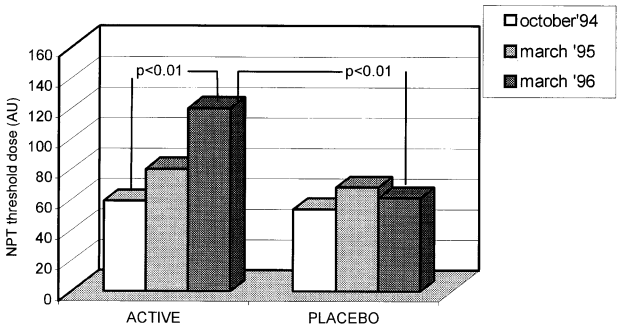

Figure 4 Mean values of threshold doses of NPT performed before starting the treatment (October 1994), after five months (March 1995) and at the end of treatment (March 1996). A significant increase of NPT threshold dose (AU) was evident at the end of the trial in the active group ($P<0.01$), with a significant difference from the placebo group ($P<0.01$).

documented. The ease of use and lack of severe side-effects may constitute a relevant prerequisite for further studies needed to define if this immunotherapeutic approach has value in special populations, as well as children and adults with mild symptoms, or in the primary prevention of respiratory allergic diseases.

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