

Local nasal immunotherapy in allergic rhinitis to *Parietaria*

A double-blind controlled study

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Preseasonal local nasal immunotherapy (LNIT) by means of an extract in macronized powder form has been studied in allergic rhinitis to *parietaria*. Twenty-four *Parietaria*-sensitive patients have been studied for 18 weeks in a double-blind controlled trial. Subjects were selected on the basis of a positive skin test, RAST and intranasal challenge to *Parietaria* antigen. Three eight-patient groups were randomly planned: the first group was given native *Parietaria* product, the second modified *Parietaria* product, and the third placebo. During the pollen season no difference was observed in mean weekly symptom score between the three groups, while the mean weekly medication score was significantly lower in the treated groups than the control group. Only the treated groups showed a significant increase in specific nasal threshold to *Parietaria* after treatment. Adverse reactions to LNIT, limited to the upper respiratory tract, occurred rarely and did not interfere with the dose schedule. This study indicates that LNIT in powder form may be a suitable alternative to the traditional subcutaneous immunotherapy in terms of clinical efficacy and safety.

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Parenteral immunotherapy (PIT) for seasonal rhinitis has been proven effective in many controlled trials (23); however, it has some disadvantages, including cost, compliance, and, though rare, severe anaphylactic reactions (15).

PIT generally produces a systemic rather than a local immune response (6, 32). Studies using viral vaccines have shown that local immunization of mucous membrane induces a distinct, protective local immune response, characterized by an increase in specific secretory IgA-antibodies that is not seen after parenteral immunization (24). According to these concepts, local nasal immunotherapy (LNIT) represents an attempt to induce an immune tolerance, directly at the organ site of an allergic disease, by repeated inhalations of the causative allergen. Its possible efficacy has been demonstrated by reduction of sensitivity to grass pollen in patients repeatedly challenged intranasally with increasing doses of allergen in nasal provocation tests (31). For several years LNIT has been tried as an experimental treatment program mostly for ragweed-induced allergic rhinitis. In experimental studies, however, intranasal spraying of unmodified aqueous extracts has caused immediate rhinitis symptoms (19, 34).

Therefore, more recent studies have used chemically modified allergens, which have a reduced allergenicity while still retaining immunogenicity (5, 8, 10, 14, 19, 22). These studies have shown good clinical results, rare local adverse effects, and a local production of IgA and IgG following the treatment. A recent development in LNIT as regards stability and standardization is the introduction of allergen lyophilized in macronized powder form (24). Controlled studies with this kind of LNIT in adults (25) and in children (4) have shown good clinical results and tolerability in grass-pollen-induced rhinitis. In our study we evaluated the efficacy of a similar treatment in allergic rhinitis to *Parietaria* pollens, one of the major causes of pollinosis in our area.

Material

Subjects

Twenty-four subjects (13 females and 11 males), aged between 14 and 45 years, suffering from allergic oculo-rhinitis for at least 2 years were selected (Table 1). Criteria for inclusion were:

a) typical history of *Parietaria*-allergic rhinitis;

Table 1. Demographic data

	Placebo -treated	Parietaria -treated	Modified parietaria -treated
Patients <i>n</i>	8	8	8
Mean age (yr $\pm$ SD)	27.8 ($\pm$ 9.6)	31.7 ($\pm$ 14.1)	31.5 ($\pm$ 8.8)
Sex (M/F)	4/4	3/5	4/4
Years of disease ($\pm$ SD)	4 ($\pm$ 1.8)	3.1 ($\pm$ 2)	3.8 ($\pm$ 2.9)

- b) 3⁺–4⁺ positive skin test (29) to *Parietaria* extract in glycerinated solution (5% p/w) (Lofarma Allergeni-Milan, Italy);
- c) RAST positivity (at least class 2);
- d) positive response to specific nasal provocation test;
- e) informed written consent.

Patients were excluded for the following reasons: 1) asthma, 2) chronic use of nasal corticosteroids and/or decongestants, 3) perennial rhinitis, 4) prior specific immunotherapy, 5) other allergen sensitivity, 6) pregnancy or lactation, 7) nasal polyps.

Antigen

Two active products (kindly supplied by Laboratorio Farmaceutico Lofarma, Milan, Italy), native and modified, were used for the study. The native product (Pa) was prepared from *Parietaria judaica*-*P. officinalis* (50-50 mix) pollen extract, standardized in allergenic units (AU) by a RAST inhibition technique (1) in comparison with in-house standard. Titration in international units (IU) was not possible because the *Parietaria* International Reference Standard is not yet available. The Pa extract was incorporated into an inert excipient mass (lactose) and dehydrated. For particle size analysis, the powder was suspended in silicone oil, sonicated and analyzed with a laser light-powered (wavelength 632.8) analyzer (Cilas mod. 715, France): 70% of particles were 25–80 μ m; mean value 45 μ m. Hard gelatine capsules were filled with 25 mg of the powder, at increasing doses: 2.5 – 5 – 10 – 20 – 40 – 60 – 80 – 120 – 160 – 240 AU. The powder was administered into the nostrils with a manual nasal insufflator (MIAT, Milan, Italy).

The modified product (PaM) was prepared from the *Parietaria judaica*-*P. officinalis* extract previously described, and modified by a patented chemical procedure (Italian patent No. 48431 A/89 and its international extension).

Briefly, this comprised the following steps:

- the extract was gel-filtrated on Sephadex® G-25, and the excluded peak, corresponding to the Blue dextran standard, collected;
- K cyanate 0.5 M (final) and sodium tetraborate 0.1 (final) were added to the extract, the pH ad-

justed to 9.3 with 0.1 M NaOH, and the mixture incubated at 40°C for 20 h;

- the sample was left at room temperature for 1 h and rechromatographed as described above.

The modified product was tested for allergenic activity, in parallel with the non-modified extract as control, by the RAST-inhibition procedure according to Anderson & Baer (1), with the following modifications:

- polystyrene beads, 6.4 mm in diameter, rough surface, were used as solid support reagent (Sferikit®, Lofarma, Milan);
- the ¹²⁵I-anti-IgE from goat was from a commercial source (Sferikit®, Lofarma, Milan);
- a pool of 70 *Parietaria*-positive sera (RAST class 3 or 4) was used as IgE source.

Statistical validation of data and the 50% inhibiting-dose (C₅₀) calculation was done according to the above-mentioned reference (1).

The modified product (PaM) proved to be 400 times less allergenic than the non-modified extract, the C₅₀ values being 3.90 μ l and 0.0098 μ l, respectively.

Incorporation of the extract into lactose powder, particle size analysis, capsule filling, and administration of the product were performed as previously described for the native product (Pa).

The placebo consisted of lactose and in exterior was identical to the active products.

Methods

Sera, collected in October and November 1988, were assayed for IgE antibodies to *Parietaria* by Sferikit® RAST (Lofarma, Italy). Results were calculated as percentages of radiolabeled ¹²⁵I-anti-IgE bound.

Pollen counts were made daily by a pollen trap, Burkard type. *Parietaria* pollen counts were > 30 grains/m³ during a period of 59 days, from 28 April to 26 June: the peak pollen period.

LNIT was self-administered; it was preseasonal, beginning in December 1988 and lasted for 18 weeks, according to two schedules (Table 2):

- 1) The first schedule (13 weeks) consisted of administration of increasing doses of the allergen by a special device, alternate day alternate nostril. Each patient received cromolyn (5.2 mg) 15–30 min before treatment. To avoid progression of the allergen through the bronchial tree, patients were told to vocalize during each administration. The schedule comprised the following doses: 2.5 – 5 – 10 – 20 – 40 – 60 – 80 – 120 – 160 – 240 AU. Each dose was administered four Times;
- 2) The second schedule (maintenance period) was carried out for 5 weeks; each patient was treated with maximal tolerated doses (240 UA in 14 patients, 120 in 2 patients) twice weekly.

Table 2. Dosage schedules

Week	Allergenic units administered
Increasing doses period	
1-2	25
3-4	85
5-6	260
7-8	500
9-10	960
10-12	1360
Maintenance period	
13-14	960
15-16	960
17-18	960

Experimental design

The patients were randomly divided into three groups according to randomization tables (2): Group 1 (8 subjects) = placebo; Group 2 (8 subjects) = active *Parietaria*-extract (Pa); Group 3 (8 subjects) = active modified *Parietaria*-extract (PaM).

Before and after each treatment, the following tests were performed (Fig. 1):

- skin reactivity threshold assessment by skin prick test with *Parietaria* extracts in decreasing concentrations, according to "end-point method"; all patients were tested with the following dilutions: 2%, 1%, 0.5%, 0.1% w/v. The wheals were outlined with a fine-point marker and transferred via transparent tape onto permanent records. The areas were measured by planimetry and expressed in mm². The end-point is the highest dilution eliciting > 3 mm wheal (17);
- non-specific bronchial provocation test with methacholine, in order to exclude an important non-specific bronchial hyperreactivity;
- nasal challenge tests: nasal sensitivity was determined by quantitative challenges with increasing concentrations of allergen in powder form (2.5 – 5 – 10 – 20 – 40 – 60 – 80 AU) which were

delivered, as during treatment, by a manual rhino-insufflator (26). Threshold doses of allergen were determined from the amount required: a) to increase total nasal resistance to airflow of 50% or more, as compared with the inert control using anterior rhinomanometry (RN4 Rhinomanometer – Markos, Monza, Italy) (3); b) to elicit at least two of the four symptoms: itching, sneezing, discharge and obstruction (28).

To assess the reproducibility of this specific nasal provocation test we compared, outside the *Parietaria* season, two specific challenges at a 1-week interval in 10 randomly chosen study patients. The threshold doses obtained from the two challenge sessions correlated closely ($r = 0.874$, $p \leq 0.0005$).

Clinical evaluation

Both LNIT-treated and control patients kept a daily report of allergic symptoms during the treatment and during the pollen season. Daily symptom-medication scores (SMS) were recorded for each patient. Specific symptoms recorded were nasal itching, nasal obstruction, sneezing, running nose, eye irritation and watering. Patients graded each symptom daily for severity from 0 to 4 (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe). The amount of antihistamines (terfenadine 60 mg) used was also recorded. The patients were instructed to use this drug when needed.

The patient self-evaluation was made at the end of the study, before the code was broken. Each patient was asked for his/her overall evaluation of this season, as compared with previous seasons, under categories of not improved, improved, or asymptomatic.

Statistics

The differences between symptom and pharmacological scores in the active- and placebo-treated

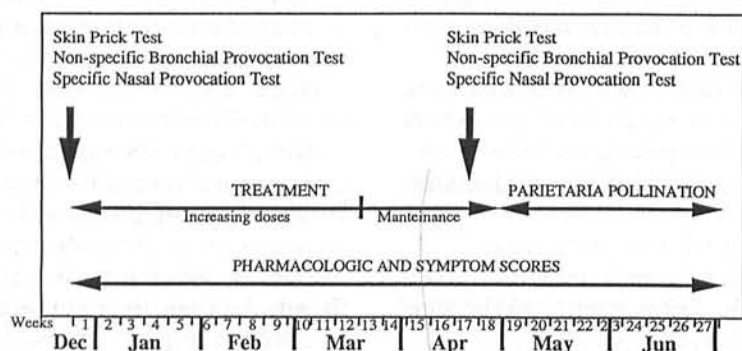


Fig. 1. Study design.

groups were calculated by Student's t-test (2-tailed). The chi-square test was used to test the significance of the differences among the overall evaluations expressed by each patient at the end of the study.

Results

Two patients of the control group withdrew from the study, one owing to transportation problems and, the other left the area during the pollen season.

Clinical response

Adverse reactions during LNIT were mild rhinorrhea, occasional sneezing and slight nasal congestion immediately after use of the nasal spray. These symptoms occurred only with the higher dosages (160 and 240 AU) in four patients (three in Pa-group and one in PaM-group). These side-effects rarely lasted more than 30 min. There were no systemic reactions, but these minimal local adverse reactions did not interfere with the dose schedule.

The mean weekly symptom scores for each group are shown in Fig. 2. These were calculated by summing the individual symptom scores for each patient for each week and dividing by the number of patients within that group. During treatment no significant difference in mean weekly symptom scores occurred among the three groups, although symptom scores were slightly higher in the actively treated groups. During the pollen season this parameter did not differ significantly in the three groups (Fig. 2), with wide fluctuations according to the pollen count. However, the mean weekly medication scores differed significantly during the pollen season between the Pa-treated group (4 weeks) and the PaM-treated group (5 weeks), as compared with the control group (Fig. 3).

Statistical significance was not reached when the contingency table analysis was performed among the

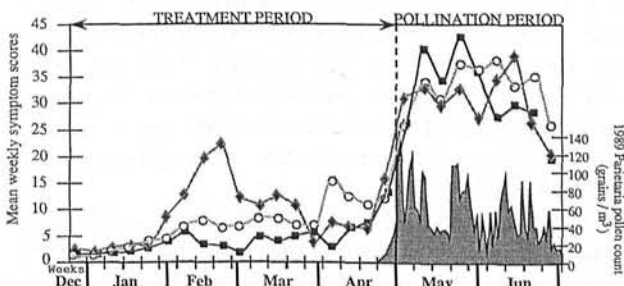


Fig. 2. Mean weekly symptom scores in Pa-treated (○), Pa M-treated (○·) and placebo (■) groups. The daily *Parietaria* pollen counts are shown in the shaded area. The treatment period is shown by the double arrow (May 1 to July 2). There were no differences in symptom scores between the three groups.

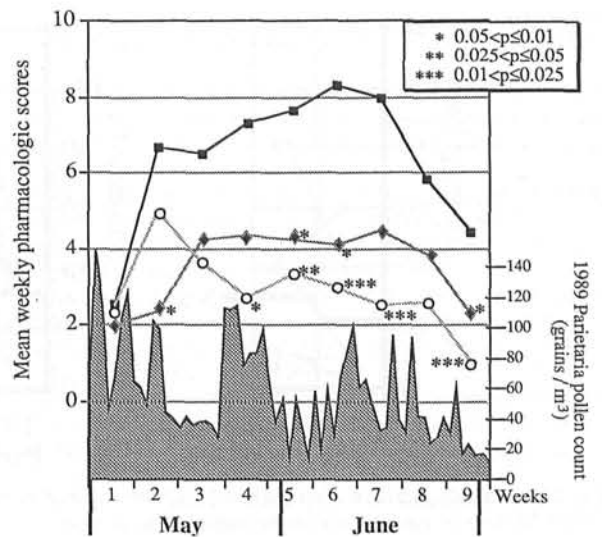


Fig. 3. Mean weekly medication scores during the pollination period in Pa-treated (○), Pa M-treated (○·) and placebo (■) groups. Daily *Parietaria* pollen counts are shown in the shaded area.

Table 3. Comparison among patients' evaluations about improvement achieved with treatment

	Not improved	Improved	Asymptomatic
Placebo-treated	4	2	0
Parietaria-treated	1	2	5
Modified <i>Parietaria</i> -treated	1	2	5

Total $\chi^2 = 8.556$ $0.05 < p \leq 0.1$

overall evaluations from the patients finishing the trial (Table 3).

Nasal provocation test

After treatment, nasal sensitivity to *Parietaria* pollen measured by NPT significantly decreased in the actively treated groups, while no difference was observed in the control group (Fig. 4). No significant differences between the three groups were observed regarding skin reactivity and bronchial hyperresponsiveness before and after treatment.

Discussion

Previous studies demonstrated that LNIT with aqueous ragweed extract is clinically effective in alleviating rhinitis symptoms in a dose-dependent manner (7, 33, 36); however, higher doses of the aqueous extract cause significant local adverse reactions, thereby limiting its usefulness (19, 21, 35). Low doses of aqueous ragweed extract minimize these side-effects but are not clinically effective (9, 33, 36). Other authors have used a polymerized ragweed extract

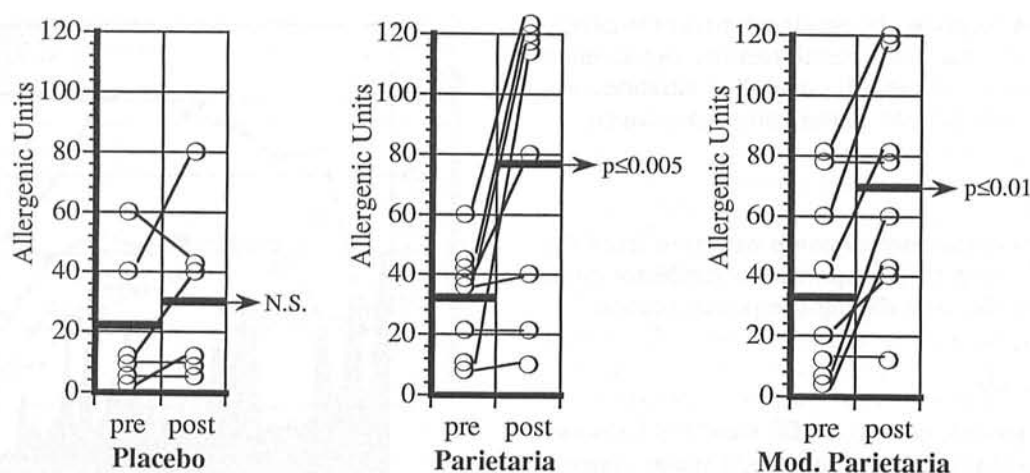


Fig. 4. Threshold doses of each patient (○) before and after LNIT in the three study groups. Horizontal bars (—) indicate mean threshold dose of each group before and after treatment.

(PRW) in order to avoid or reduce local side-effects. A better clinical response with minimal side-effects was observed in patients treated only with high doses of PRW, confirming that the effectiveness of LNIT is dose-dependent (22, 33, 36). Similar results were reported in grass-pollen rhinitis, using grass allergens modified with glutaraldehyde (5, 8, 14, 27). In agreement with these results in a multicentre, double-blind study of grass-pollen-allergic rhinitis in children (4) and adults (25), LNIT with allergen extract in powder form showed significant clinical efficacy and good tolerability.

In our study LNIT against *Parietaria*, using allergen extract in powder form, appeared clinically effective and well tolerated. Although the reduction in nasal symptoms during the pollen season did not differ significantly among the three groups, significantly fewer antihistamines were used in the treated groups than in the control group. Moreover, the nasal specific threshold increased significantly only in the treated groups. No difference was observed regarding symptom scores and drug consumption during treatment among the three groups, even if symptom scores were lower in the placebo group.

No asthmatic attacks occurred during the treatment, confirming that macronized *Parietaria* extract does not cause bronchoconstriction in allergic rhinitis (11). The low-dose sodium cromoglycate, used as premedication, must, in our opinion, be considered negligible as a therapeutic measure.

The mechanism underlying the clinical benefits of such a treatment is not clear; however, three mechanisms can be suggested:

1) *A local immunological response.* Local immunotherapy has been shown to induce local immunological responses. Some authors have noted a significant increase of nasal specific IgA and IgG, but

clinical and immunological findings do not correlate (10, 12, 16, 20, 21, 22, 33, 36).

2) *Systemic immunological response.* Patients treated locally with aqueous extract showed a measurable systemic immunological response, i.e. a marked increase in serum-IgE during the treatment period which was not followed by the usual coseasonal increase (19). On the other hand, Welsh et al. noted no change in serum IgE and IgG when using aqueous extract (34). In a recent study (18) clinically relevant allergenic activity in the systemic circulation has been revealed by a sensitive modified RAST (immuno-capture RAST) in animal models after intranasal treatment with allergen in powder form. Nevertheless, the quantity of allergen able to reach the systemic circulation is lower than that obtained by means of subcutaneous immunotherapy.

3) *Induction of a specific tolerance.* Passive antigenic stimulation of the respiratory mucosa may serve a protective function. It was shown in rats that repeated inhalation of aerosols containing ovalbumin or ragweed antigen induced a transient IgE response which switched off spontaneously, despite continuing aerosol exposure. Subsequent challenge of the animals revealed that aerosol exposure had selectively suppressed responsiveness to these antigens in the IgE class, without affecting corresponding IgG responses (7, 13, 30).

In conclusion, we propose LNIT as a safe alternative to the traditional subcutaneous treatment. However, further investigations on a wider population are needed to clarify its mechanism, the most suitable length of treatment, the persistence of therapeutic effect after stopping treatment, and its clinical efficacy in comparison with traditional immunotherapy.

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