

Long-term follow-up of nasal immunotherapy to *Parietaria*: clinical and local immunological effects

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Summary

Background Local nasal immunotherapy (LNIT) with extracts in powder has been demonstrated clinically effective and devoid of side-effects in several controlled trials; nevertheless, no data concerning the long-term effects of LNIT are presently available.

Methods In a recent double-blind, placebo-controlled study of LNIT to *Parietaria* pollen we observed, by means of specific nasal provocation test (SNPT) that LNIT is able to modify the local allergic inflammatory response. In the present study we followed up the same patients in open fashion for 2 further years.

Results The results confirmed the clinical efficacy of LNIT and showed that it is strictly dependent on pre-seasonal administration: in fact, after LNIT discontinuation a clinical relapse was observed. A certain long-lasting protective effect on SNPT parameters (nasal symptoms and neutrophils infiltration) was also observed, whereas an increase of eosinophils count and ICAM-1 expression on nasal epithelial cells appeared as possible markers of clinical relapse.

Conclusion The present study suggests that pre-seasonal LNIT can be taken in consideration in selected subjects as prophylactic treatment for pollen-induced rhinitis. In addition, the results obtained provide informations about the duration of clinical efficacy and add data about the local allergic inflammation and its modulation.

Keywords: local nasal immunotherapy, allergic inflammation, specific nasal provocation test

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Introduction

Local nasal immunotherapy (LNIT), was introduced in clinical practice on empirical basis, with the aim of achieving a target organ desensitization in allergic rhinitis and of making the treatment more convenient and acceptable to patients [1]. Some early controlled studies [2–6] demonstrated the clinical efficacy of LNIT, although troublesome local effects were sometimes present. In the last 10 years, new powdered extracts appeared to be devoid of remarkable side-effects, still yet maintaining a satisfactory clinical effectiveness [7–13]. Recently, our group demonstrated, by means of specific nasal provocation test (SNPT), a

significant effect of pre-seasonal LNIT to *Parietaria* on local allergic inflammation in a double-blind placebo-controlled study [12]. The data provided in our previous study, suggested some questions [14], mainly concerning the model employed, the possible chance to predict the clinical efficacy of LNIT and the relationship between the duration of the treatment and its effectiveness. Only recently, some data about long-term effect of subcutaneous immunotherapy [15,16] have been provided, but such data still lack for LNIT. The present long-term follow-up of our previous study provided answers to some of the mentioned questions. After double blind key revealing the formerly placebo treated patients switched to LNIT before 1994 pollen season and before 1995 pollen season none of the two groups received LNIT. We monitored both clinical scores under natural exposure and the inflammatory response to

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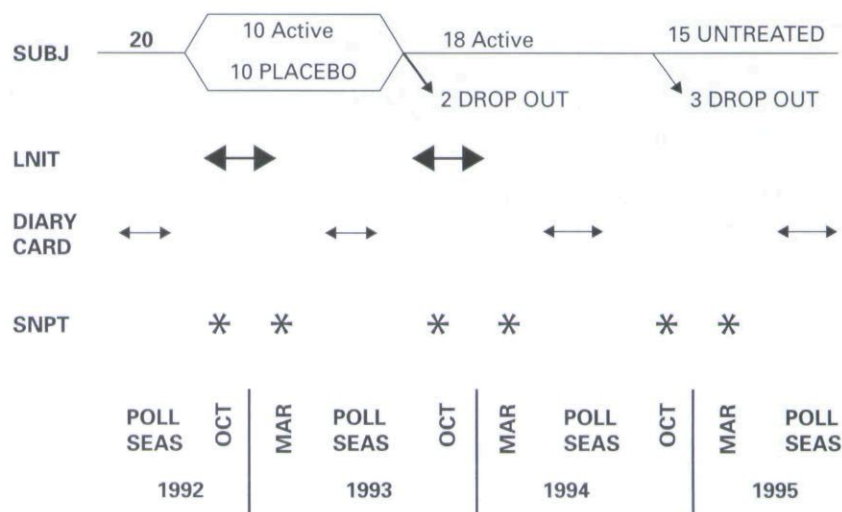


Fig. 1. Study design. LNIT, local nasal immunotherapy; SNPT, specific nasal provocation test.

nasal challenge. LNIT showed a certain protective effect on nasal challenge parameters up to 12 months after its discontinuation. On the other hand, a clinical relapse upon seasonal pollen exposure was observed after LNIT suspension. Moreover, an increased ICAM-1 expression on epithelial nasal cells appeared as predictive marker of a possible clinical relapse.

Methods

Study design

The double-blind phase of the study has been previously described [12]. Briefly: after a baseline clinical evaluation of symptom/medication scores during the 1992 pollen season (April–June) patients were randomized into placebo and active group and clinical scores were reassessed during 1993 pollen season. The double-blind key was revealed in November 1993, then both groups received active treatment and clinical scores were recorded again in 1994 pollen season. LNIT treatment was then stopped in both groups and scores were assessed in 1995 season. SNPT was performed, every 6 months, since October 1992 (t0) until march 1995 (t30). The study design is reported in Fig. 1.

Patients

Twenty patients (11 men, nine women, mean age 33 years) with single sensitization to *Parietaria* pollen were enrolled. All patients suffered from rhinoconjunctivitis and five out of them also suffered from mild intermittent asthma. All patients subscribed an informed consent and the study was approved by the Department Ethical Committee.

Treatment

LNIT (Allerkin, Laboratorio Farmaceutico Lofarma, Milan)

was administered pre-seasonally as capsule-coated dried powder, titred in allergenic units. Each increasing dose (2.5, 5, 10, 20, 40, 60, 120, 160 AU) was assumed twice weekly for 2 weeks, then a maintenance dose of 240 AU was assumed once a week. LNIT administration was preceded by nasal cromolyn, and patients were required to vocalize during insufflation.

Diary card

A daily record of symptoms and drug intake was kept by each patient during the pollen seasons (April–June) 1992, 1993, 1994, 1995. Symptoms (nasal blocking, rhinorrhoea, itching, sneezing, conjunctivitis, cough, asthma) were graded as follows: 0 = absent, 1 = mild, 2 = severe. Drug intake (beta2 agonists, antihistamines, cromolyn, local/systemic steroids) and side-effects due to LNIT were recorded as well.

Specific Nasal Provocation Test

SNPT was performed out of pollen seasons, being the patients symptom-free, every 6 months: t0 (October 1992), t6 (March 1993), t12 (October 1993), t18 (March 1994), t24 (October 1994), t30 (March 1995). All medications were withdrawn 10 days before SNPT (30 days for astemizole or inhaled steroids). The SNPT technique has been described elsewhere [12,17]. The threshold provocative dose was established for each patient at the beginning of the study and the inflammatory parameters were assessed 30 minutes after provocation. We considered: nasal symptoms score; neutrophils and eosinophils count in nasal scraping cytopins (expressed as mean of 10 fields in light microscopy); ICAM-1 positivity score on epithelial nasal cells (graded from 0 to 4) assessed by immunoenzymatic alkaline phosphatase-anti alkaline phosphatase procedure [12,17,18].

Statistical analysis

The two groups of patients were kept separated after double-blind key revealing, in order to better monitor the considered parameters in relation to the duration of the treatment. Thus, the labels 'placebo group' and 'actively treated group' were maintained. Symptoms/medication scores, cell counts and ICAM-1 positivity were assumed as non-parametrically distributed: Wilcoxon test (with Dunn's post-test correction) for intragroup comparison, and Mann-Whitney *U*-test for intergroup (unpaired/unmatched samples) comparison were used.

Results

Two patients dropped out in the first year, as previously reported. Three patients, two from the active and one from the formerly placebo group, dropped out in March 1995, after the last SNPT, because of loss of interest. Thus, we could analyse SNPT data of 18 (nine active and nine placebo) patients until t30, while we had available the clinical scores of 15 (seven active and eight placebo) patients in the 1995 pollen season. No significant side-effect due to LNIT was experienced by the patients during the whole study period.

Symptoms/medications score

The score was calculated as sum of the monthly scores (April & May & June). The pollen counts of the considered seasons were quite similar (Fig. 2). The clinical scores of the two groups were homogeneous during the run-in (1992) pollen season. The active group showed a significant improvement after the first course of treatment (1992 vs 1993; $P=0.01$) and this improvement was maintained after the second course (1992 vs 1994 $P=0.007$; 1993 vs 1994; $P=NS$). The placebo group showed no clinical score modification in the first year, while a significant improvement was observed after switching to active treatment (1992 vs 1994; $P=0.01$; 1993 vs 1994; $P=0.01$). After treatment suspension, a clinical worsening was observed in both groups (1994 vs 1995; $P=0.05$; 1992 vs 1995; $P=NS$). The intergroup comparisons are consistent with these findings, as summarized in Fig. 2.

Specific nasal provocation test

We have examined the following inflammatory parameters.

Nasal symptoms In the double-blind phase, nasal symptoms improved only in the active group (t0 vs t6; $P=0.007$; t0 vs t12; $P=0.003$). This improvement vs baseline persisted at t18, t24 and t30. After transferring to active treatment, also the formerly placebo group showed an

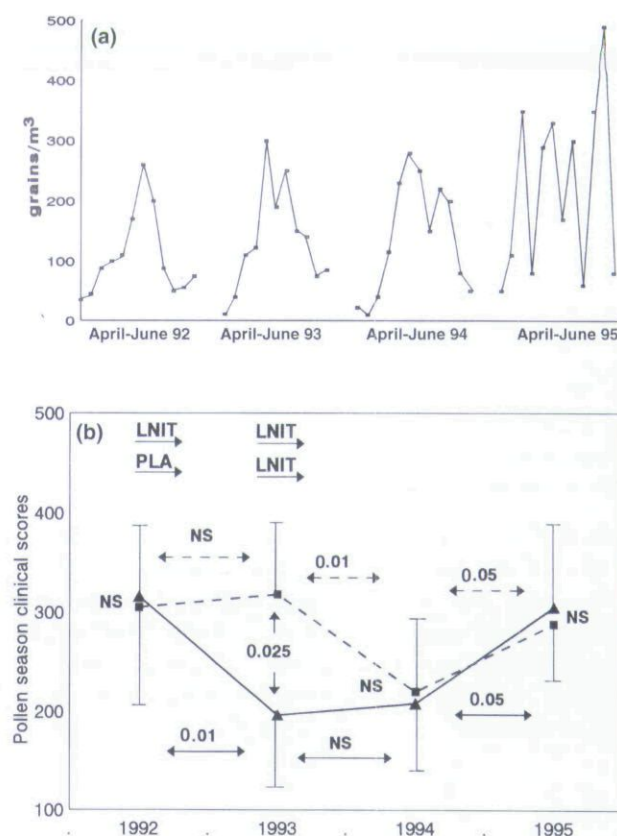


Fig. 2. (a) *Parietaria* pollen counts during the considered seasons. (b) Symptoms/drug intake score (Mean, sd). Inter-group and intragroup comparisons are shown in the graph. The kind of treatment administered (active LNIT or PLAceto) is reported upon the graph.

improvement of nasal symptoms (t12 vs t18; $P=0.01$; t12 vs t24; $P=0.01$). The symptoms improvement persisted in both groups even at t30, 12 months after LNIT discontinuation (t0 vs t30 $P=0.04$), as reported in Fig. 3.

Neutrophils A similar behaviour was observed for neutrophils count in nasal scraping (Fig. 4). After switching to active treatment, neutrophils decreased in the formerly placebo group (t12 vs t18; $P=0.01$; t12 vs t24; $P=0.007$). The neutrophils count reduction persisted up to t30 vs t0 in both groups ($P=0.04$).

Eosinophils Eosinophils count in nasal scraping decreased in the active group (t0 vs t12 $P=0.003$) and subsequently also in placebo group after LNIT (t12 vs t18; $P=0.01$; t12 vs t24; $P=0.02$). The reduction was maintained up to t24. Interestingly, after LNIT discontinuation, an increase of eosinophils count was observed both in active and

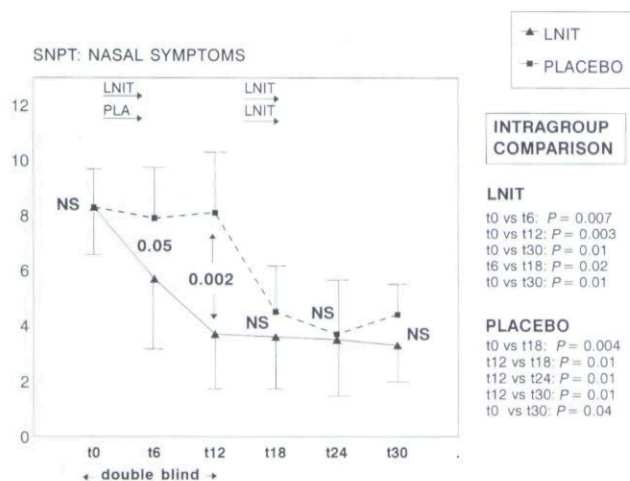


Fig. 3. SNPT Symptom score (Mean, SD). Inter-group comparisons are shown in the graph. Only significant P values of intra-group comparisons are summarized on the right. The treatment administered (active LNIT or PLACEbo) is reported upon the graph.

placebo group (t24 vs t30: $P = 0.01$ and $P = 0.03$), as shown in Fig. 5.

ICAM-1 expression It showed a trend comparable to eosinophils count: in particular, ICAM-1 expression decreased in placebo group after switching to LNIT (t12 vs t18: $P = 0.01$, t12 vs t24: $P = 0.01$). An increase of ICAM-1 expression was evidenced at SNPT after LNIT suspension in both groups (t24 vs t30: $P = 0.05$) (Fig. 6).

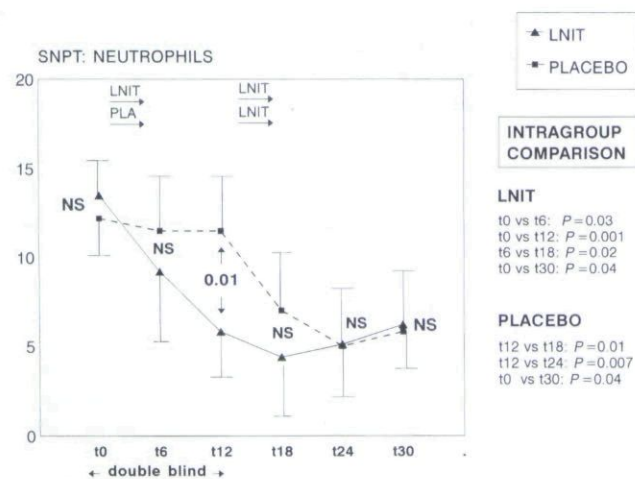


Fig. 4. SNPT: Neutrophils count (Mean, SD) in nasal scraping 30' after challenge. Inter-group comparisons are shown in the graph. Only the significant P values of intra-group comparisons are summarized on the right side. The treatment administered (active LNIT or PLACEbo) is reported upon the graph.

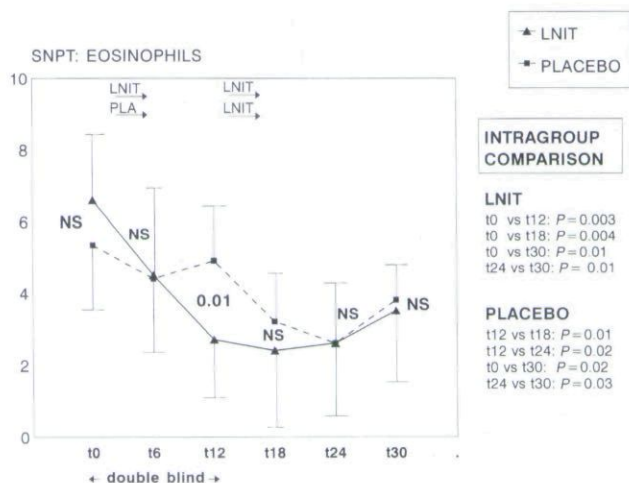


Fig. 5. SNPT: Eosinophils count (Mean, SD) in nasal scraping 30' after challenge. Inter-group comparisons are shown in the graph. Only the significant P values of intra-group comparisons are summarized on the right side. The treatment administered (active LNIT or PLACEbo) is reported upon the graph.

Discussion

The present long-term follow-up of the previous double-blind study, confirms the clinical effectiveness of LNIT in reducing clinical symptoms and drug intake under natural allergen exposure. Indeed, the number of patients (globally 15) which completed the study over a 3-year period is little, but the difficult encountered in following up such selected outcoming patients without administering a treatment, should be taken in account.

The symptoms/medication score analysis suggests that a

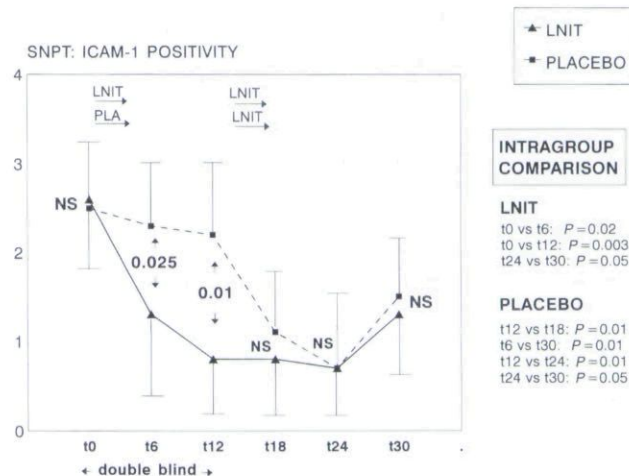


Fig. 6. SNPT: ICAM-1 positivity (Mean, SD) on epithelial cells in nasal scraping 30' after challenge. Inter-group are shown in the graph. Only the significant P values of intra-group comparisons are summarized on the right side. The treatment administered (active LNIT or PLACEbo) is reported upon the graph.

pre-seasonal LNIT treatment is required. LNIT appears to exert a protective effect on natural allergenic exposure only if administered pre-seasonally and after its discontinuation a clinical relapse is observed. The SNPT performed at 6 month intervals, provided further interesting data. Firstly, a certain protective effect of LNIT on nasal symptoms and neutrophil infiltration upon specific challenge persisted up to 12 months after LNIT suspension. Secondly, ICAM-1 expression and eosinophilic infiltration upon SNPT increased after LNIT discontinuation. Thus, these two parameters could be regarded as predictive markers of the clinical effectiveness. Furthermore, the present follow-up highlights the difference existing between natural allergenic exposure and SNPT.

Finally, LNIT should not be considered as alternative to pharmacological treatment, but complementary to it, since LNIT is able to reduce medications intake and symptoms. In this light the possible use of LNIT to treat polysensitized rhinitis patients in association to subcutaneous immunotherapy to other allergens may be envisaged. In addition, the socioeconomical save [19] due to the self-administration of treatment (i.e. absence of the costs of the injectory administration), should be taken in consideration. Indeed, the administration technique require a careful choice of the patients to treat: adult, well-trained subjects, suffering from rhinitis only, and/or showing low compliance with subcutaneous immunotherapy.

Although further studies are required in order to optimize schedules and dosages, and to assess the effectiveness of other allergenic extracts, both the available studies and the present result can suggest LNIT as an effective desensitization tool.

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