

Pharmacokinetics of radiolabelled Par j 1 administered intranasally to allergic and healthy subjects

G. Passalacqua*, V. Altrineti†, G. Mariani‡, P. Falagiani§, G. Mistrello§, R. Brizzolara†, G. W. Canonica* and M. Bagnasco†

*Allergy & Respiratory Diseases, †Medical & Radiometabolic Therapy, DIMI, University of Genoa, Genoa, Italy, ‡Nuclear Medicine, University of Pisa (formerly Nuclear Medicine, University of Genoa), Pisa, Italy and §Lofarma S. p. A., Milan, Italy

Summary

Background Local nasal immunotherapy is accepted as an alternative to the injection route for allergic rhinitis. Despite this, little is known about the kinetics of the allergen after nasal delivery in allergic subjects.

Objective We aimed at assessing the biodistribution of ^{123}I -radiolabelled Par j 1 in *Parietaria*-allergic subjects, in comparison with healthy volunteers.

Methods Purified Par j 1 was radiolabelled with ^{123}I and sprayed into the nostrils of three control subjects and three *Parietaria*-allergic volunteers. Dynamic and static scintigraphic images of the head were recorded at serial times and blood samples were obtained to measure the plasma radioactivity, and to assess the presence of circulating radiolabelled species by gel chromatography.

Results In *Parietaria*-sensitized subjects, the radiolabelled allergen was rapidly cleared from the nasal cavity and transported to pharynx, and little local persistence was seen. This differed from healthy subjects where nasal clearance of the tracer was slower and nasal radioactivity persisted up to 24 h. The increase in plasma radioactivity paralleled swallowing of the allergen in both groups, and plasma chromatographic profile did not differ between allergic and healthy volunteers.

Conclusions Sensitization to the allergen affects its local biodistribution. Gastrointestinal absorption is relevant also for the intranasal route.

Keywords allergic rhinitis, local nasal immunotherapy, Par j 1, pharmacokinetics, radiolabelling

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Introduction

Local nasal immunotherapy (LNIT) is presently accepted for clinical use in the official documents [1–3]. The rationale for giving immunotherapy nasally is based in part on the observation that repeated stimulation of the nasal mucosa with low doses of allergen results in a hypo-responsiveness [4]. This can be easily demonstrated and quantified by sequential nasal challenges [5], and represents an opposite phenomenon of the well-known priming effect.

Allergens for LNIT are usually prepared as aqueous solution (or as powder-filled capsules) to be sprayed into the nose through a proper device. The administration requires good training and some precautions. In fact, there is in principle the risk of inhalation of the allergen into the deep airways. There are numerous randomized placebo controlled trials (for a review, see [6]), showing the efficacy of LNIT in allergic rhinitis (AR) because of pollens and mite, and some data about its immunological effects are also available [7, 8].

Concerning the long-lasting effects, one trial suggested that LNIT has to be administered every season [8], whereas another trial showed that clinical benefits persist for 4–5 years after discontinuation [9]. Overall, in the last years, the clinical use of LNIT is progressively declining in favour of sublingual immunotherapy (SLIT), since this latter is much easier to manage and is effective also in patients with asthma. Nevertheless, LNIT remains a viable alternative to subcutaneous immunotherapy in adult, well-trained patients with pollen-induced rhinitis only, and especially in patients refusing injections [3].

Similar to other local routes of immunotherapy administration, modality of absorption, fate and distribution are expected to be crucial elements affecting the mechanism of action and the clinical efficacy of LNIT. Despite this, very few data are available in animals and humans. We have developed a nuclear medicine technique to study the biodistribution of allergens administered via mucosal routes. By means of that technique we previously assessed, for the first time in humans, the biodistribution and pharmacokinetics of radiolabelled Par j 1 in healthy volunteers [10]. In the present work we provide further data, obtained in both healthy subjects and volunteers with AR because of *Parietaria judaica*. These data may have relevant clinical implications, notably under the point of view of the bioavailability of the allergen after administration.

Correspondence: Giovanni Passalacqua, Allergy & Respiratory Diseases, Department of Internal Medicine, Padiglione Maragliano, L. go R. Benzi 10, 16132 Genoa, Italy.
E-mail: passalacqua@unige.it

Material and methods

We studied the pharmacokinetics of the radiolabelled Par j 1 administered intranasally to three adult allergic volunteers and three healthy subjects, using the same experimental protocol. The procedure, described in detail in the previous pharmacokinetics study [10], is summarized below.

The three allergic volunteers had rhinitis solely induced by *Parietaria*, confirmed by the clinical history of seasonal rhinitis and by skin test positivity to *Parietaria* (weal > 5 mm) alone. They were studied during winter, being free of symptoms and medications. None of the studied subjects had nasal abnormalities (polyposis, septal deviation, turbinate hypertrophy) and had not suffered from cold symptoms in the last 2 weeks. Routine laboratory parameters, free thyroid hormones and thyrotropin, were assessed before the study. Thyroid uptake of radioiodine was minimized by giving a potassium iodide solution (10 drops three times daily in the 3 days preceding the study) and potassium perchlorate (400 mg on the day of the study). Each subject was exposed to a radioactivity dose that was 1/10 of a standard chest radiograph, therefore the study protocol was approved by the inner Ethical Committee. All volunteers signed an informed consent form prior to the study.

The Par j 1 allergen (supplied by Lofarma S. p. A., Milan, Italy) was obtained by extraction in a 90% (NH₄)₂SO₄ solution, followed by repeated chromatographs to achieve a single peak with molecular weight of 12.5 kDa [11]. The allergen, at concentration of about 800 µg/mL, was stored in 0.15 mol/L phosphate-buffered saline. The allergen was radiolabelled by the Iodo-gen method [12] with 30–40 µg of Par j 1 and 200–300 MBq of carrier-free ¹²³I (Amersham Health, Saluggia, Italy). Unreacted ¹²³I-iodide (20–50%) was then removed by gel filtration through Sephadex G-25 M pre-packed PD-10 columns (Pharmacia, Uppsala, Sweden). ¹²³I was chosen for his short physical half-life of 13.2 h and its γ-ray emission, that provides optimal external detection with a low exposure.

The final ¹²³I-Par j 1 preparation had an average specific radioactivity of about 3.7 MBq/µg. The allergen was always administered within 60 min after labeling. The dose (about

10 µg Par j 1) was sprayed into a nostril in 50–70 µL volume, while the subject hold breath. A nose grip was then immediately placed. The subject had to stay close to the gamma camera collimator for 20–30 min without swallowing, while images were acquired. Scintigraphic acquisitions were carried out with a large-field-of-view gamma camera (model SP6; Elscint, Haifa, Israel). Early scintiscan of head and neck (sequential images, 1 image/15 s) were recorded continuously up to 30 min after the administration, with the subject's head still and close to collimator. Subsequently, static scintigraphic images were recorded at various time-points (30 min, 1, 2, 3, 5 and 18–24 h) after the administration.

Heparinized 5 mL venous blood samples were collected at 5, 10, 20, 30, 60, 120 and 180 min during the study. One-milliliter plasma aliquots were prepared and counted in a γ-counter for radioactivity content. The plasma samples obtained after 1 and 2 h after allergen administration were chromatographed by gel filtration with PD-10 columns (Sephadex G-25 M).

Results

After the allergen administration the allergic volunteers reported nasal itching and perception of rhinorrhoea, but the nasal grip prevented them from sneezing. No other relevant side-effect was observed during the experiments. Figures 1 and 2 show the sequence of scintiscan acquisitions in the first 30 min after administration of the tracer in one allergic and one healthy subject respectively as an example. This behaviour was the same in all the volunteers. Radioactivity accumulated in the nose during the very first minutes, but it rapidly moved towards the upper pharynx and disappeared almost completely within 30 min in the allergic volunteers. In parallel, a rapid decrease of intranasal radioactivity was documented. This differed from what observed in healthy controls (Fig. 2). In this latter case, although a progression of the radioactivity towards upper pharynx was apparent, radioactivity persisted at nasal level until the end of the dynamic scintiscan. Such a different behaviour between allergic and normal individuals was consistently observed in all the experiments performed. Accordingly, looking at the

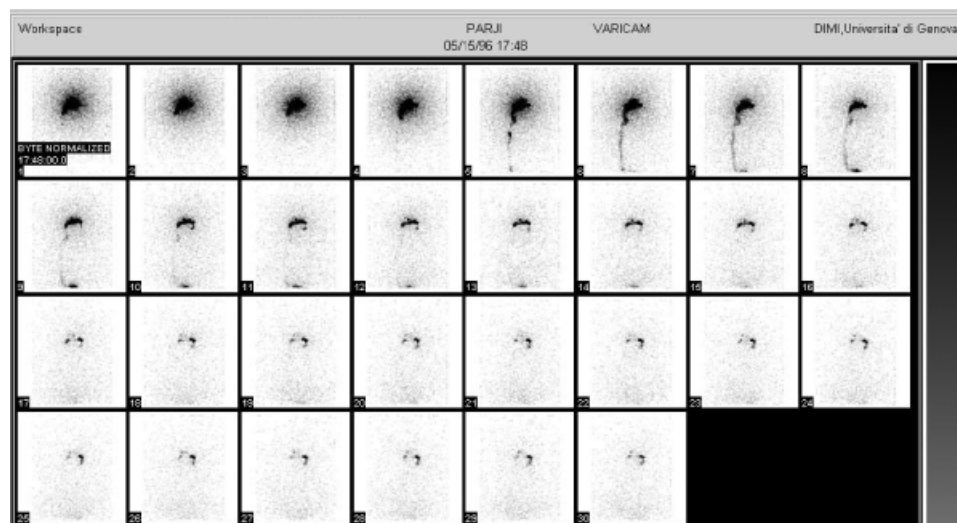


Fig. 1. Sequence of scintiscan acquisitions (1 frame/min) up to 30 min after the nasal administration of the labelled allergen in an allergic volunteer.

late scintiscan (Fig. 3), a persistence of radioactivity up to 24 h in the nose could be observed in normal individuals, but not at all in allergic subjects.

The plasma radioactivity profiles were closely similar in allergic and non-allergic individuals. As previously reported [10] plasma peak was attained between 1 and 2 h after administration (Fig. 4). Similarly, gel chromatography profiles invariably showed a small peak in the region of the native allergen, whereas the majority of radioactivity was represented by free iodide and small degradation products, consistent with extensive gastrointestinal processing (data not shown).

Discussion

Intranasal allergen immunotherapy has been proposed as a local treatment for AR, and almost all the clinical trials confirmed its efficacy on rhinitis symptoms [6]. The data on local absorption and pharmacokinetics of allergens in general are, however, few and often controversial. In humans, the nasal permeability to macromolecules has been studied with ^{125} Iodine-labeled albumin, and significantly ($P < 0.001$) larger amounts of albumin-bound radioactivity were detected in the plasma of patients with atopic rhinitis compared with that of

normal subjects after 30 min [13]. In the same study, the transport of intact albumin across the nasal mucosa was demonstrated in 3/9 normal subjects, 1/9 patients with extrinsic asthma and 9/10 patients with atopic rhinitis. The question if the increase in nasal transmucosal transport observed in such patients was the cause or the effect of atopic rhinitis remained unsolved, but the ability of macromolecules (albumin has a molecular size much larger than allergens from pollens or mites) to pass across the nasal mucous membrane was demonstrated. Moreover, in rats and rabbits a significant absorption of the allergen through sublingual and nasal mucosae has been reported [11]. Nevertheless, as previously observed [10, 14], no relevant absorption of the allergen through mucosae to the bloodstream seems to occur in humans. Using a previously established technique we investigated in humans the biodistribution of a radiolabelled allergen administered intranasally. Par j 1 is one of the major allergens of the plant *Parietaria judaica* (wall pellitory), belonging to Urticaceae family, which is largely present in southern Europe where it represents a nearly perennial allergenic source [15, 16]. LNIT with *Parietaria* extracts has been the subject of clinical trials [17]. The Par j 1 is a 13 kDa glycoprotein, with a compact structure because of four disulphide bounds, and behaving as a profilin [18]. The data obtained in the present study show that most part of the administered allergen is anyway swallowed and absorbed through gastrointestinal tract in both allergic and healthy volunteers, and this explains the similarity of plasma radioactivity curves in both groups. The absorption through the gastroenteric mucosa is supported by the slow increase in plasma radioactivity, which parallels the disappearance of the radiolabelled allergen from the nose. This fact is *per se* not unexpected, given that mucociliary clearance is very active in the nasal cavities [19]. Nevertheless, a marked difference was invariably observed, between normal and allergic individuals, in the local persistence of radiolabelled allergen: radioactivity at nasal level disappeared quickly in allergic subjects, whereas it persisted for hours in normal individuals. This difference could not be observed with the sublingual administration [10, 14].

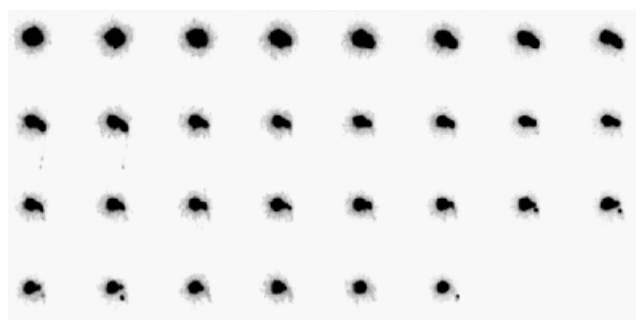


Fig. 2. Sequence of scintiscan acquisitions (1 frame/min) up to 30 min after the nasal administration of the labelled allergen in a healthy subject.

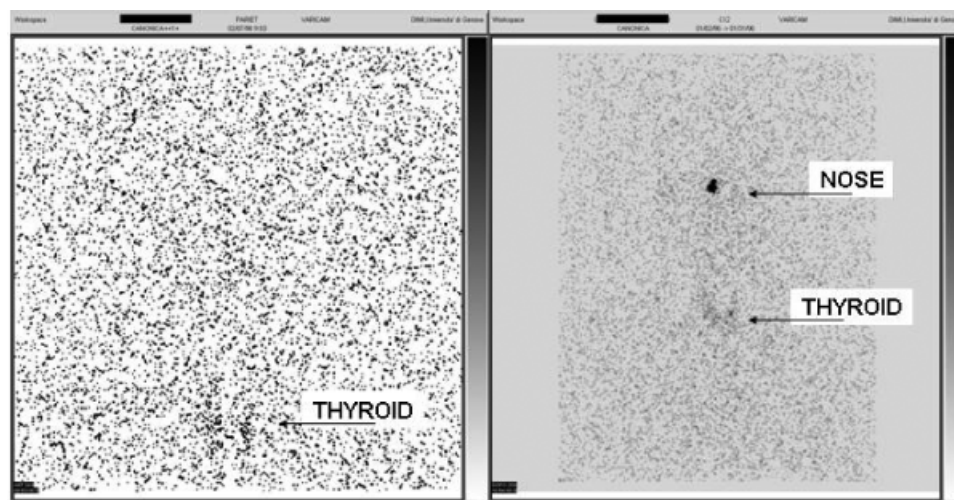


Fig. 3. Static scintiscan of the head and neck 24 h after the intranasal administration in an allergic (right) and a healthy (left) subject. Some nasal persistence of the allergen is detectable only in the non-allergic volunteer. Despite the thyroid-blocking therapy, there is some uptake of iodine by the thyroid gland.

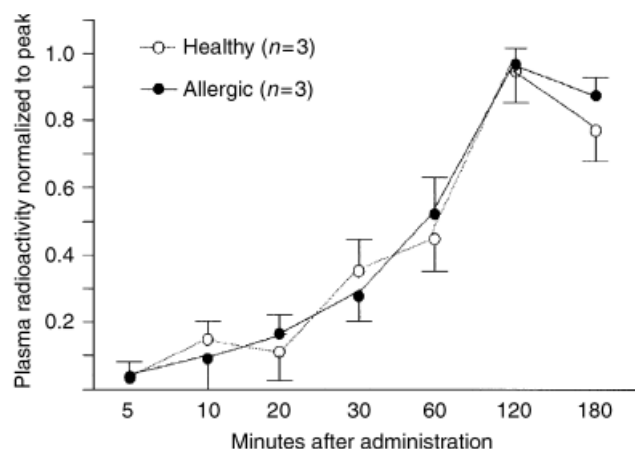


Fig. 4. Average plasma radioactivity curves (mean $\pm$ SEM, normalized to peak) in healthy and allergic subjects.

The difference observed between normal and allergic is presumably related to a faster mucosal clearance of the allergen, because of local inflammation with vasodilation, increased permeability and increased secretion rate. Although the allergic subjects were studied out of pollen season, it is likely that an IgE-mediated reaction occurred immediately after the administration of the allergen, with increased mucosal secretion, as demonstrated in nasal challenge procedures [20–22]. Of note, local radioactivity persistence is really because of the allergen, and not to free radioiodine. In fact, parallel experiments with intranasal free radioiodine failed to show the persistence of radioactivity [10]. Looking at clinical trials it is clear that the contact with mucosae is critical for the effects of local immunotherapy. In fact, when the allergen is immediately swallowed (pure oral route) the efficacy is negligible, whereas the sublingual route works. Nevertheless, in this study no persistence of the radiolabelled allergen in the nose of allergic volunteers was detectable at 24 h, and this is conflicting with the ascertained clinical efficacy. This discrepancy can be partly explained by two considerations. First, in this study a single administration is performed, whereas in clinical practice LNIT involves multiple and frequent administrations. Second, there is an intrinsic limitation related to safety, that impose the use of a low radioactivity dose, so that low amounts of persisting allergen cannot be detected. Finally, the number of studied subjects is low, but the objective difficulties in performing studies with radioactive materials has to be taken into account and, moreover, all the previous studies were performed with a similar number of patients.

In conclusion, sensitization to the allergen affects at some extent its biodistribution and kinetics, especially at mucosal level. Moreover, also in the case of nasal administration, the gastroenteric absorption has a not negligible role.

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References

- Bousquet J, Lockey R, Malling HJ, eds. World Health Organization Position Paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. *Allergy* 1998; 54 (Suppl.):2–18.
- Malling HJ, ed. EAACI Position Paper on local immunotherapy. *Allergy* 1998; 53:933.
- Bousquet J, Van Cauwenberge P, eds. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001; 108 (Suppl. 12):S83–5.
- Taylor G, Shivalkar E. Changes in nasal airways resistance on antigenic challenge in allergic rhinitis. *Clin Allergy* 1971; 1:63–73.
- Ciprandi G, Ricca V, Landi M, Passalacqua G, Bagnasco M, Canonica GW. Allergen specific nasal challenge: response kinetic of clinical and inflammatory events to rechallenge. *Int Arch Allergy Immunol* 1998; 115:157–61.
- Canonica GW, Passalacqua G. Non injection routes for immunotherapy. *J Allergy Clin Immunol* 2003; 111:437–48.
- Giannarini L, Maggi E. Decrease of allergen-specific T-cell response induced by local nasal immunotherapy. *Clin Exp Allergy* 1998; 28:404–12.
- Passalacqua G, Albano M, Pronzato C, Riccio A, Falagiani P, Canonica GW. Nasal immunotherapy to *Parietaria*: long term follow up of a double blind study. *Clin Exp Allergy* 1997; 27:904–8.
- Andri L, Falagiani P. Symptomatic relief after grass nasal immunotherapy: lasting efficacy after 4–5 years. *J Invest Allergol Clin Immunol* 2003; 13:228–31.
- Bagnasco M, Mariani G, Passalacqua G et al. Absorption and distribution kinetics of the mayor *Parietaria* allergen administered by noninjectable routes to healthy human beings. *J Allergy Clin Immunol* 1997; 100:121–9.
- Mistrello G, Rapisarda G, Falagiani P. Detection of IgE-binding activity in serum after intranasal treatment of normal rabbits with *P. judaica* extracts. *Allergy* 1991; 46:52–5.
- Fraker PJ, Speck JC. Protein and cell membrane iodination with a sparingly soluble chloramide, 1,3,4,5-tetrachloro-3a, 6a-diphenylglycouril. *Biochem Biophys Res Commun* 1978; 80:849–57.
- Buckle FB, Cohen AB. Nasal mucosal hyperpermeability to macromolecules in atopic rhinitis and extrinsic asthma. *J Allergy Clin Immunol* 1975; 55:213–21.
- Bagnasco M, Passalacqua G, Villa G et al. Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers. *Clin Exp Allergy* 2001; 31:54–60.
- D'Amato G, Spieksma FT, Liccardi G et al. Pollen-related allergy in Europe. *Allergy* 1998; 53:567–78.
- Liccardi G, Visone A, Russo M, Saggese M, D'Amato M, D'Amato G. *Parietaria* pollinosis: clinical and epidemiological aspects. *Allergy Asthma Proc* 1996; 17:23–9.
- Andri L, Senna GE, Betteli C et al. Local nasal immunotherapy in allergic rhinitis to *Parietaria*. A double-blind controlled study. *Allergy* 1992; 47:318–23.
- Asturias JA, Gomez-Bayon N, Eseverri JL, Martinez A. Par j 1 and Par j 2, the major allergens from *Parietaria judaica* pollen, have similar immunoglobulin E epitopes. *Clin Exp Allergy* 2003; 33:518–24.
- Soane RJ, Carney AS, Jones NS et al. The effect of the nasal cycle on mucociliary clearance. *Clin Otolaryngol*. 2001; 26:9–15.
- Mori S, Fujieda S, Kimura Y, Takahashi N, Saito H. Nasal challenge activates the mucociliary transport system on not only the ipsilateral but also the contralateral side of the nose in patients with perennial allergic rhinitis. *J Otorhinolaryngol Relat Spec* 2000; 62:303–6.
- Ciprandi G, Ricca V, Pronzato C, Passalacqua G, Bagnasco M, Canonica GW. Allergen-specific challenge induces intercellular adhesion molecule 1 (ICAM-1 or CD54) on nasal epithelial cells in allergic subjects. Relationships with early and late inflammatory phenomena. *Am J Respir Crit Care Med* 1994; 150:1653–9.
- Litvyakova LI, Baraniuk JN. Nasal provocation testing: a review. *Ann Allergy Asthma Immunol* 2001; 86:355–64.