

Absorption and distribution kinetics of the major *Parietaria judaica* allergen (Par j 1) administered by noninjectable routes in healthy human beings

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Background and objective: The clinical effectiveness of noninjectable routes for specific immunotherapy has been demonstrated in many studies, but no data are available on the kinetics of allergens administered by these routes. Therefore we studied the kinetics of the radiolabeled purified major *Parietaria judaica* allergen (Par j 1) after sublingual, oral, and intranasal administration to healthy human beings.

Methods: After tracer administration (10 to 12.5 µg of Par j 1 labeled with iodine 123) to nonallergic volunteers, scintigraphic images were recorded at various times. Blood samples were also obtained at serial intervals to evaluate the absorption and distribution of radioactivity in plasma and to identify circulating radioactive species by molecular exclusion gel chromatography.

Results: When the sublingual route was used, no circulating radioactivity was detected until the tracer was kept under the tongue. The labeled allergen was rapidly degraded and absorbed in the gastrointestinal tract after swallowing. Plasma radioactivity peaked at about 1.5 to 3 hours and was mostly represented by free radioiodine and small radiolabeled peptides. Some activity not caused by free ¹²³I remained associated with the oral mucosa up to 18 to 20 hours after administration. When the oral route was used, the results were similar to those observed after swallowing the sublingually administered allergen but without any persistence of the tracer in the mouth. When the intranasal route was used, the pattern of plasma radioactivity mimicked that of the sublingual and oral routes, with absorption of activity from the radiolabeled allergen occurring in the gastrointestinal tract after transport to the pharynx by mucociliary clearance. A relevant fraction of the tracer was retained on the nasal mucosa up to 48 hours after administration.

Conclusion: The data in this study provide the first experimental basis for exploring the in vivo kinetics of allergen administered through noninjectable routes for specific immunotherapy in human beings. (*J Allergy Clin Immunol* 1997;100:122-9.)

Key words: Oral immunotherapy, sublingual immunotherapy, local nasal immunotherapy, iodine 123, radiolabeling, Par j 1

Abbreviations used

¹²³ I-Par j 1:	Iodine 123-radiolabeled Par j 1 allergen
LNIT:	Local nasal immunotherapy
SLIT:	Sublingual immunotherapy

Allergen-specific immunotherapy is currently used in the treatment of respiratory allergy. Subcutaneous immunotherapy is burdened with a certain risk of severe side effects,¹⁻³ and for this reason, over the last 20 years some noninjectable routes for immunotherapy administration have been proposed and developed: oral immunotherapy, sublingual immunotherapy (SLIT), and local nasal immunotherapy (LNIT). The aim of these routes is to achieve an effective hyposensitization combined with improved safety and acceptance to the patients.⁴ In oral immunotherapy treatment the allergenic extract is swallowed, in SLIT the preparation is kept under the tongue without swallowing for at least 1 minute, and in LNIT the allergenic extract is sprayed into a nostril during vocalization. Many double-blind controlled studies have proved the clinical effectiveness of noninjectable immunotherapy for several allergens⁵⁻¹⁸; oral immunotherapy also appears capable of evoking systemic immunologic changes^{5, 7, 9, 10} resembling those induced by subcutaneous immunotherapy, whereas LNIT mainly exerts its effects locally.^{13, 14, 17}

The mechanisms of action of subcutaneous immunotherapy have recently been partly explained,^{19, 20} and concerning the noninjectable routes, some basic immunology observations suggested the tolerogenicity of both the oral and the inhalational route.²¹⁻²⁴ Nevertheless, the mechanism of action of noninjectable immunotherapy remains unknown; moreover, different administration routes most likely involve different processing and degradation pathways of the allergen, not to mention the different types of preparation.²⁵ Only sparse data²⁶⁻²⁹ are presently available about the in vivo kinetics of allergens administered through noninjectable routes. Therefore we believe that elucidating this point would be the first step toward better understanding of the mechanisms of action of these treatments. We report the results of experiments concerning the kinetics of the major *Parietaria judaica* allergen (Par j 1) labeled with radioiodine

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in human beings. This particular allergen was chosen because *P. judaica* is a widely diffused allergen in the Mediterranean area with a long-lasting pollen season (over 6 months per year) and because the incidence of sensitization to this pollen is about 40%. The radiolabeled purified allergen was administered orally, sublingually, and intranasally to nonallergic volunteers in order to provide basic pharmacokinetic information, which would be useful for better comprehension of the mechanism of action of noninjectable immunotherapy.

METHODS

Aim of the study and overall design

This study investigated the absorption and fate of iodine 123-radiolabeled Par j 1 allergen (^{123}I -Par j 1), administered by noninjectable routes to healthy human beings. The radiolabeled allergen was given orally, sublingually, or intranasally to nine nonallergic subjects; and two subjects in this group also received a free ^{123}I dose intranasally or sublingually in order to compare the kinetics of the radioactive label per se with the kinetics of the radiolabeled allergen. The possible degradation of the radiolabeled allergen in the sublingual environment was also investigated.

Subjects

Nine healthy volunteers (4 men and 5 women) aged 27 to 47 years were enrolled for a total of 11 studies. Routine laboratory parameters were assessed in each subject before the study, including measurement of free thyroid hormones and thyrotropin plasma levels. The considered parameters were within normal range in all subjects. None of the subjects was sensitized to *Parietaria* pollen, as confirmed by skin prick test and RAST. Thyroidal uptake of radioiodine was minimized by administering a saturated potassium iodide solution (10 drops three times daily in the 5 days preceding the study) plus potassium perchlorate (200 mg twice daily for 2 days, starting on the day of the study). All volunteers signed an informed consent form, and the study protocol was approved by the Ethical Committee of the Department of Internal Medicine.

^{123}I -radiolabeled allergen

Par j 1 allergen was obtained by treating crude *P. judaica* extract with a 90% $(\text{NH}_4)_2\text{SO}_4$ solution; the supernatant was then dialyzed against 5 mmol/L NH_4HCO_3 and eluted through a high-performance liquid chromatography reverse-phase column (C18, 13 $\times$ 300 mm; Deltapack, Waters) with acetonitrile/trifluoroacetic acid (0.1%) at a flow rate of 6 ml/min. Fractions containing Par j 1 (as identified with a specific antibody) were pooled, lyophilized, and repeatedly chromatographed in the same conditions to yield a single peak with a retention time of 32.6 minutes and molecular weight of 12.5 kd (demonstrated by sodium dodecylsulfate-polyacrylamide gel electrophoresis analysis). The allergen was stored as sterile solution in 0.15 mol/L phosphate-buffered saline at a concentration of about 800 $\mu\text{g}/\text{ml}$. ^{123}I was chosen for radiolabeling because of its favorable physicochemical properties (main gamma emission, 159 keV; half-life, 13.2 hours).

The allergen was radiolabeled by the iodo-gen method³⁰ with 30 to 40 μg of Par j 1 and 185 to 300 MBq (5 to 8 mCi) of carrier-free ^{123}I (supplied by Sorin Biomedica SpA, Italy, at the specific activity of 7400 TBq/ $\mu\text{mol}/\text{L}$ or 200 Ci/ $\mu\text{mol}/\text{L}$). Unreacted ^{123}I -iodide (20% to 50%) was then removed by gel filtration through Sephadex G-25 M by using prepacked PD-10

columns (Pharmacia, Uppsala, Sweden). The final ^{123}I -Par j 1 preparation had an average specific radioactivity of about 3.7 MBq/ μg (100 $\mu\text{Ci}/\mu\text{g}$). Preliminary experiments performed with iodine 125 showed remarkable stability to deiodination of the radiolabeled allergen over several days both in physiologic saline solution and serum.

Administration procedures

Three subjects each received the radioallergen by sublingual, oral, and intranasal routes, respectively. Moreover, one subject from the sublingual route group and one from the intranasal route group also received free ^{123}I alone in order to evaluate the kinetic pattern of ^{123}I per se. Each subject received approximately 10 to 15 μg of Par j 1 purified allergen per administration, for an approximate radioactive dose of 37 to 56 MBq (1 to 1.5 mCi). The tracer was administered within 60 minutes after radiolabeling.

When the sublingual route was used, the dose was delivered under the tongue by a micropipette in a 100 to 200 μl volume. The subject was then required not to swallow or move for 30 minutes while an early scintigraphic acquisition (see below) was recorded. Subsequently, the subject was allowed to move, swallow, and rinse his or her mouth. When the oral route was used, the dose was diluted in 50 ml of water and immediately swallowed. Only single scintigraphic images were recorded. When the nasal route was used, the dose was sprayed into a nostril by a puffer in 50 to 70 μl volume, and a nose grip was then immediately applied. The subject was kept close to the gamma camera for 20 to 30 minutes while an early scintigraphic acquisition was recorded (see below). The subject was then allowed to move and to wipe his or her nose, if needed.

Scintigraphic acquisitions

A large-field-of-view gamma camera (model SP6; Elscint, Haifa, Israel) was used for scintigraphic acquisitions. For the sublingual and nasal routes, early scintigraphic acquisitions were recorded. Early scintigraphic acquisition on administration of ^{123}I -Par J 1 consisted of a series of sequential images continuously recorded at the rate of 1 image/min over 20 to 30 minutes (dynamic imaging). During such dynamic acquisitions, the subject's head was kept close to the gamma-camera collimator. After the initial dynamic imaging (and also in subjects in the oral route group in whom no dynamic acquisition was performed), single scintigraphic images (static imaging) of the head, chest, and abdomen were recorded at 30 minutes and at 1, 3, 5, and 18 to 24 hours (48 hours in selected cases) after administration of the radiolabeled allergen.

Blood sampling and determination of labeled species in plasma

Heparinized 5 ml venous blood samples were collected from each subject during the study. All plasma was separated by centrifugation, and 1 ml aliquots were counted in a well-type gamma-counter for radioactivity content. Furthermore, analysis of radioactive species circulating in plasma (for samples obtained at 1 to 3 hours after tracer administration) was performed by gel filtration with prepacked PD-10 columns (Sephadex G-25 M).

Finally, the possible degradation/deiodination of the radiolabeled allergen in saliva was investigated. A sample of saliva was collected from the mouth floor of one subject about 20 minutes after sublingual allergen administration. Gel chromatography of the sample was then performed through Sephadex G-25 M

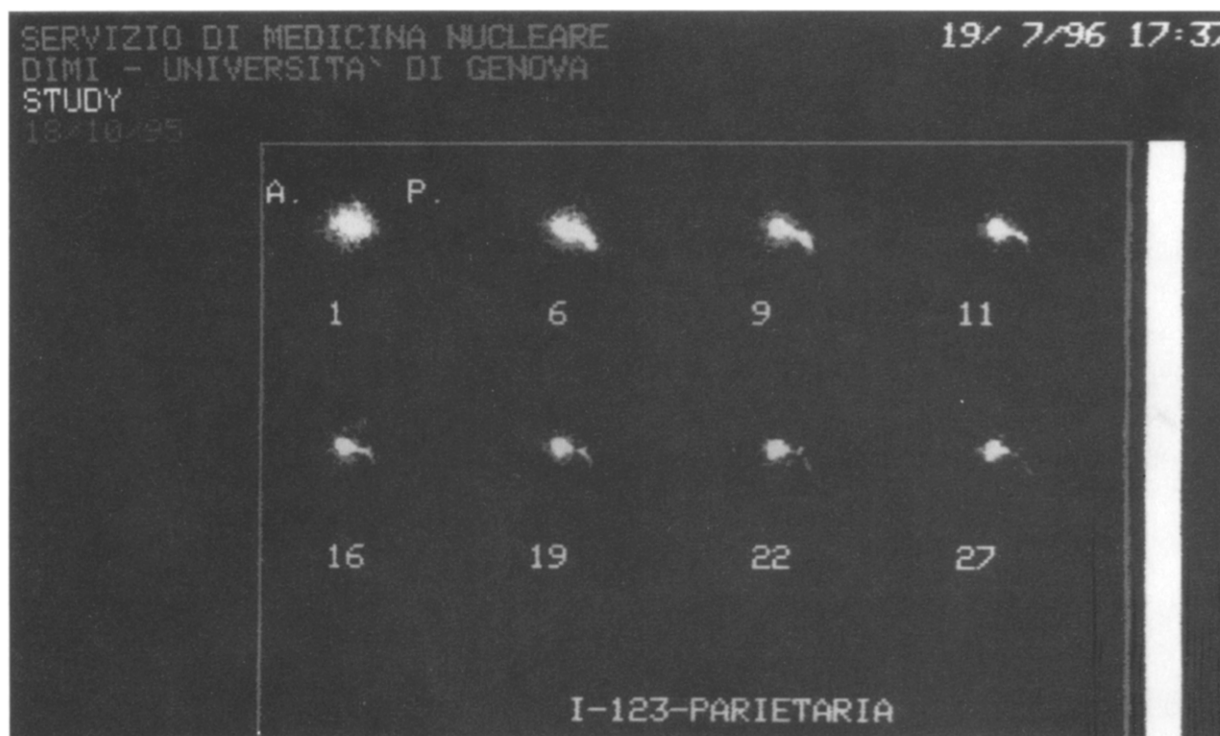


FIG. 1. Selected frames from dynamic acquisitions over 30 minutes (1 frame/min) on nasal administration of ^{123}I -Par j 1. Images are recorded in lateral projection, with anterior part of the head (A) facing left and posterior part (P) facing right. Movement of radioactivity toward the upper pharynx (as a result of mucociliary clearance) and swallowing of a sizable fraction of radioactivity are clearly visible already at 6 minutes.

columns, after saliva was added to an aliquot of serum from a donor pool.

RESULTS

No adverse event caused by the radiolabeled allergen administration was observed. Degradation/deiodination of the labeled allergen kept for 20 minutes under the tongue was minimal.

Scintigraphic imaging

Sublingual route. Dynamic imaging during the first 30 minutes after sublingual administration of ^{123}I -Par j 1 showed no change in the radioactivity content within the mouth until the dose was kept under the tongue without swallowing. This observation was confirmed by the clear-cut constant activity/time curve determined for a region of interest defined over the mouth. After swallowing, rapid distribution of the tracer in the gastrointestinal tract took place, superimposable to that observed after oral administration of any radioactive bolus. Interestingly, even after extensive mouth rinsing, a significant amount of radiolabeled allergen (about 2% of the dose) persisted at the sublingual level up to 20 hours. These kinetics significantly differed from those of free ^{123}I , which disappeared quickly from the sublingual region.

Oral route. After swallowing, rapid distribution of the radiolabeled allergen in the gastrointestinal tract was visualized by static imaging, as in all esophageal transit studies with a radioactive bolus. Small intestine ap-

peared within minutes after the stomach, followed by renal excretion of radioactivity and visualization of urinary bladder within 3 hours. No scintigraphic evidence of radioactivity accumulation at specific sites corresponding to any defined lymphatic structure was detected.

Intranasal route. Shortly after administration, a large fraction of the allergen was observed to move toward the upper pharynx (probably by means of mucociliary clearance) during dynamic acquisition. This fraction of the radiolabeled allergen was then swallowed, as shown by the scintigraphic visualization of the esophagus (Fig. 1). Movement of the allergen toward the upper pharynx was also confirmed by the radioactivity/time curves obtained during the dynamic 30-minute acquisition for two separate regions of interest, defined over the anterior nasal cavity and upper pharynx, respectively. A relevant amount of allergen (about 10% of the administered dose) persisted at the nasal level up to 40 hours after administration. This pattern of relevant retention in the nasal mucosa was not observed with free ^{123}I . Finally, no radioactivity accumulation, other than the nonspecific pattern of free iodine distribution, was ever detectable in the bronchial tree after intranasal administration.

Plasma radioactivity kinetics

Radioactivity counts of plasma samples (normalized to peak concentrations for easier comparison) are plotted in Fig. 2. The kinetics of plasma radioactivity confirm the

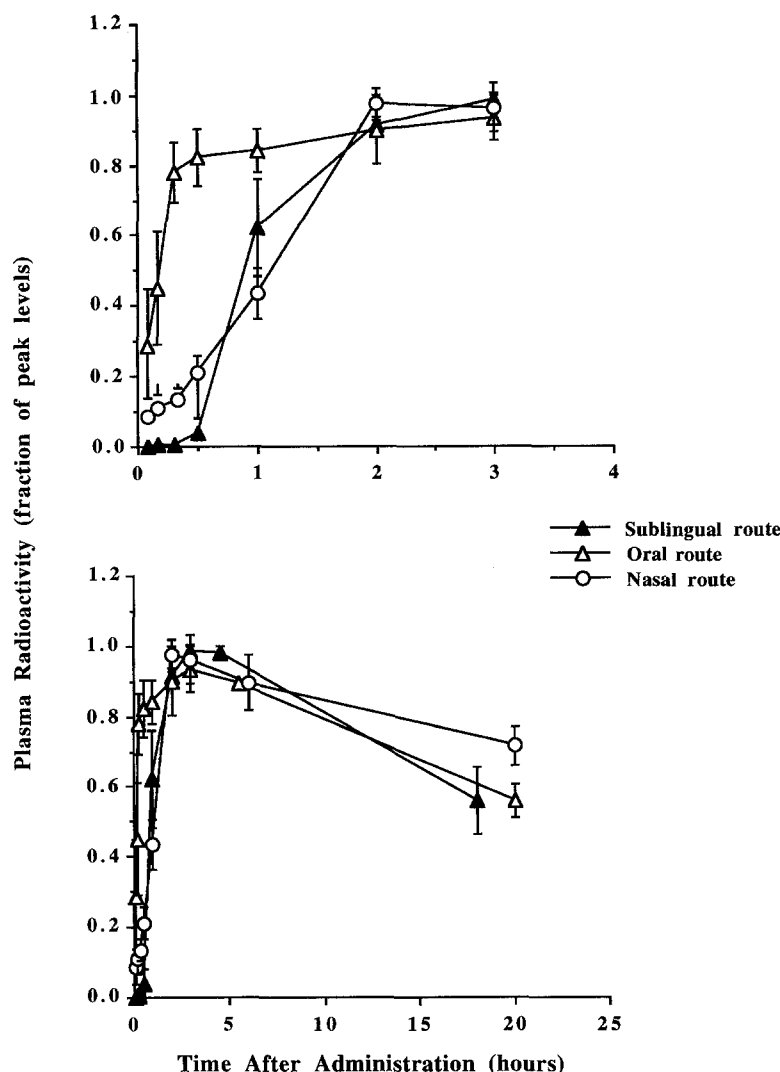


FIG. 2. Average plasma radioactivity curves (mean + SEM, normalized to peak levels) observed in three groups of healthy volunteers receiving ^{123}I -Par j 1 by sublingual, oral, and nasal routes. The detail (**upper panel**) representing early phase (from zero time to 3 hours after administration) serves to illustrate different patterns of plasma radioactivity curves observed in the three groups of subjects. Complete representation from zero time to about 20 hours after administration of ^{123}I -Par j 1 (**lower panel**) shows pattern of plasma radioactivity clearance after plateau.

observations made by means of external scintigraphic imaging. In subjects receiving the radiolabeled allergen orally, plasma radioactivity rapidly increased, peaking at about 30 minutes (Fig. 2, *top*). In subjects given ^{123}I -Par j 1 sublingually, plasma radioactivity was not detectable until swallowing. After swallowing, plasma radioactivity rose and strictly paralleled that of the oral route. In subjects given the radiolabeled allergen intranasally, plasma radioactivity progressively increased, consistent with esophageal transit and absorption through the gastrointestinal tract of the amount of allergen transported from the nose to the pharynx.

The pattern of plasma radioactivity disappearance

after peak was similar in all subjects, irrespective of the administration route (Fig. 2, *bottom*). Chromatographic analysis showed that the pattern of total radioactivity in plasma does not accurately reflect the fate of the allergen. In fact, gel filtration of plasma through Sephadex G-25 columns invariably showed a major peak in the low molecular weight region (free iodine and small peptides), as indicated in Fig. 3 (*upper panel*). As expected, deiodination and degradation of the allergen occur during the transit in the gastrointestinal tract, leading to absorption of free radioiodine and small peptides only. Consistent with these events, anion-exchange chromatography, performed on four samples from two different

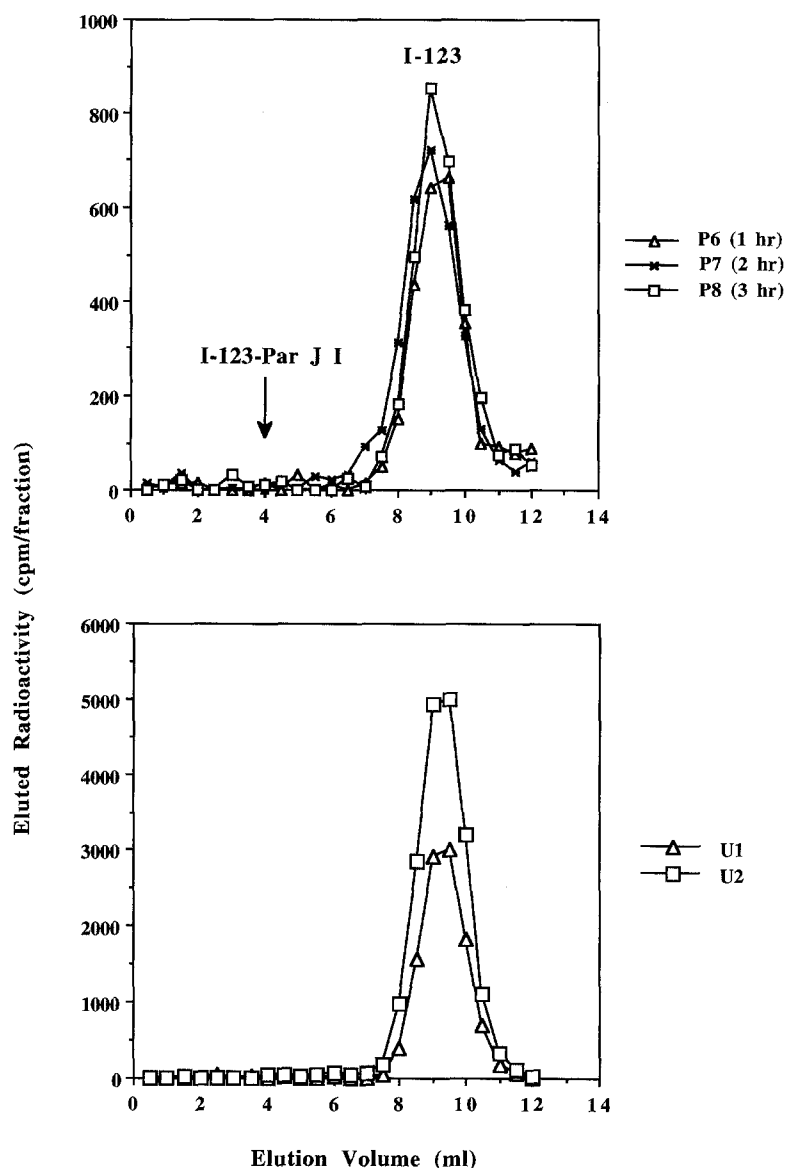


FIG. 3. Upper panel, Radioactivity elution profiles (through Sephadex G-25 columns) of plasma samples obtained in one healthy volunteer receiving ^{123}I -Par j 1 by sublingual instillation (no difference could be detected among the three groups of subjects concerning the general pattern of elution profiles). Most of radioactivity is eluted in the zone of free radioiodide or very small peptides. Small changes above background in the elution zone, corresponding to intact radiolabeled allergen (indicated in the plot), are most likely due to the matrix effect of serum (nonspecific interaction of radioiodide with plasma proteins), because they were also observed after administration of free ^{123}I -iodide. **Lower panel,** Radioactivity elution profiles (through Sephadex G-25 columns) of urine samples (collected in intervals 0 to 2 hours [U1] and 2 to 5 hours [U2], respectively) after sublingual instillation of ^{123}I -Par j 1. Single peak can be seen in the elution zone of free ^{123}I (between 7.5 and 12 ml) without any activity eluted in the zone of the intact allergen (between 2.5 and 6 ml).

individuals, demonstrated that about 10% of radioactivity was bound to peptides (not shown). Thus there was no direct evidence of diffusion of the intact allergen through the mucosae.

Gel chromatography of urine samples invariably showed the presence of a single peak in the elution zone of radioiodide without any radioactivity eluted in the allergen zone (Fig. 3, lower panel). This observation is most likely due to absence of the serum protein

matrix effect in the case of urine samples. Furthermore, comparative plasma disappearance rates of radioallergen and of free iodine in two individuals (Fig. 4, upper panel), showed a more rapid disappearance of the free label, consistent with the presence of circulating radiolabeled peptides in subjects given the radioallergen and/or with slower gastrointestinal absorption of radioactivity when ^{123}I -Par j 1 was administered.

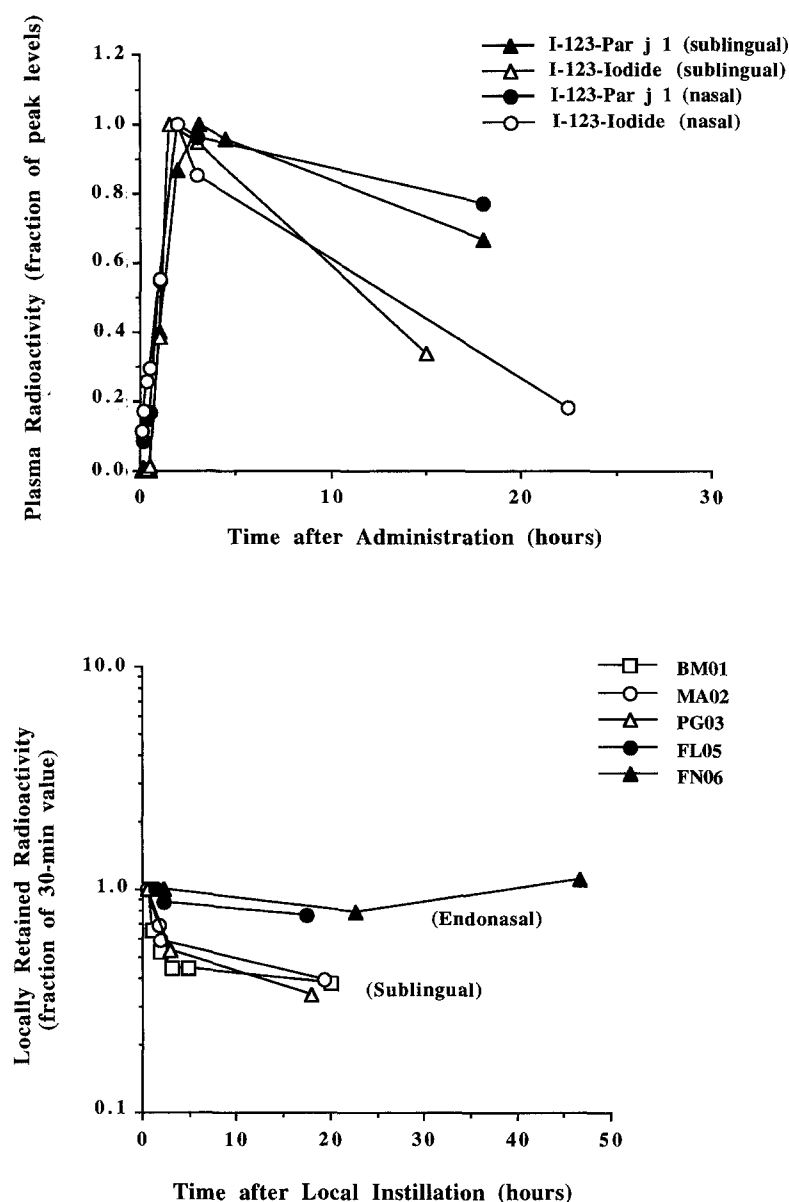


FIG. 4. Upper panel, Plasma radioactivity curves observed in two healthy volunteers receiving nasal or sublingual ^{123}I -Par j 1 (filled symbols) and in the repeat study (each in the same subject) with nasal or sublingual free ^{123}I -iodide (open symbols). Clearance of radioactivity from plasma is much faster after administration of free radioiodide than after administration of radiolabeled allergen, suggesting that plasma radioactivity in subjects receiving the allergen includes some slow-clearing radioactive species (possibly represented by peptides originated from in vivo degradation of administered allergen). Lower panel, Local retention of radioactivity after nasal (filled symbols) and sublingual (open symbols) instillation of ^{123}I -Par j 1, calculated on scintigraphic regions of interest at different times. Each symbol indicates an individual experiment in a single subject.

DISCUSSION

The effectiveness of noninjectable specific allergen immunotherapy has been demonstrated in several clinical studies.⁵⁻¹⁸ This study was carried out to investigate the kinetics of distribution of a radiolabeled allergen administered through different noninjectable routes in healthy human beings, because the only available in vivo data concerning this issue have been obtained in animal models. Thus the

experimental approach in this study is intended to provide an experimental basis for understanding the relationship between route of administration and mechanisms of action of noninjectable routes. The labeling procedure we used yielded minimal degradation of the protein. In fact, preliminary labeling experiments with ^{125}I (half-life, 60 days) demonstrated that the radiolabeled allergen is stable over several days of incubation.

Furthermore, no degradation of the radiolabeled allergen was observed to occur in the sublingual space up to 20 minutes. Therefore within certain time limits, radionuclide imaging is likely to reflect the distribution of the radiolabeled allergen, at least before massive degradation and deiodination occur in the stomach.

The first noteworthy observation was the complete absence of absorption of the radiolabeled allergen through the sublingual mucosa to the bloodstream up to 30 minutes; in this regard, it must be pointed out that patients treated with SLIT usually keep the allergen under their tongues for 1 to 2 minutes. This scintigraphic and radiopharmacokinetic finding is consistent with the large size and relatively high molecular weight of the protein, which also possesses a hydrophilic surface. Consistently, plasma radioactivity increased only after swallowing. On the other hand, in subjects receiving the radiolabeled allergen orally, plasma radioactivity started to rise within 10 minutes. Some clinical implications can be derived from these data: in fact, our findings show that the hypothesized risk of a too rapid absorption of the allergen through the sublingual mucosa^{4,9} is devoid of any experimental basis. In addition, a fraction of the radiolabeled allergen persists at the sublingual level (Fig. 4, *lower panel*) for a long time, far longer than free radioiodine administered as control. It is possible to speculate that such persistence plays some role in the mechanism of action of SLIT, although it is not possible to clarify the exact localization of the allergen within the mucosal structures on the basis of scintigraphic images alone; it is possible that slow absorption and processing of the locally retained allergen occurs through the local immune system.

As far as nasal administration is concerned, it is obvious that a large part of allergen is swallowed after passive transport to the pharynx by ciliary clearance; in this regard, the pattern of plasma radioactivity is consistent with the imaging data. Also in this case, persistence of the radiolabeled allergen at the nasal level was long-lasting (up to 48 hours), which is in agreement with the described clinical evidence¹⁷ for a local effect. Furthermore, no bronchial deposition of the allergen has been observed after nasal administration; this minimizes the likelihood of risk of asthma attacks caused by the nasal administration of immunotherapy, as previously prospected.⁴ Furthermore, it is conceivable that the immunologic modifications recently observed during LNIT by Romagnani et al. (personal communication), namely reduction of allergen-specific proliferative response of T cells induced by natural allergen exposure, are related, at least in part, to the allergen absorbed in the gut.

The data from this study only represent a preliminary step toward the comprehension of the mechanisms of action of noninjectable routes for immunotherapy; however, some general conclusions can be drawn, and further research plans can be explored. First, gastrointestinal absorption of the degraded allergen appears, in the experimental conditions of this study, to be the most

relevant event. Allergen deiodination/degradation significantly challenge the study of postabsorption kinetics, as demonstrated by gel filtration chromatography: the disappearance of plasma radioactivity was slower after administration of ¹²³I-Par j 1 than after administration of free iodine, which is consistent with the presence of circulating small radiolabeled peptides and presumably with ongoing radioactivity transfer across the mucosae (slower for the allergen than for the label). High-performance liquid chromatography analysis of species circulating in plasma may provide more detailed information about these peptides and their kinetics. Second, the persistence (Fig. 4, *lower panel*) of a fraction of the radiolabeled allergen at the site of administration (sublingual and nasal) suggests some local processing of the antigen. In this regard, it would be of interest to study the allergen's kinetics in patients with allergy, both those with symptoms and those free of symptoms. This could provide interesting information about the relationships existing between local absorption and individual sensitization or local inflammation. This aspect is of relevance, because it has been reported³¹ that patients with allergy have a more rapid allergen absorption through nasal mucosa than normal subjects. Finally, similar studies with different allergens are necessary to achieve more general conclusions.

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