

Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers

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Summary

Background and objective Little is known about the pharmacokinetics of allergens for local immunotherapy. Thus, we studied the pharmacokinetics in allergic volunteers of a commercial allergenic vaccine in orosoluble tablets (LAIS®, Lofarma S.p.A.).

Methods The carbamylated monomeric allergoid derived from *Parietaria judaica* major allergen (Par j 1), characterized by maintenance of the original molecular size, and the native allergen, were radiolabelled with ¹²⁵I, then incorporated into the commercial soluble tablets and administered to allergic subjects. Early sequential and late static scintigraphic acquisitions were performed, and plasma radioactivity was measured at different time intervals.

Results No difference in local pharmacokinetics was observed between the allergen and the allergoid: part of the tracer was retained in the mouth for at least 2 h after swallowing. No direct absorption through the oral mucosa could be detected, as plasma radioactivity increased only after swallowing and peaked at 2 h. However, the plasma peak attained with the allergoid in tablets was significantly higher with respect to the native allergen. Finally, some undegraded allergoid, but not the allergen, could be constantly detected in the bloodstream at plasma peak.

Conclusions The results showed a similar behaviour of the allergoid and the allergen in tablets as far as their local kinetics are concerned, whereas plasma peak was higher with the allergoid than with the allergen. Therefore we conclude that the chemical modification of the allergen may affect its pharmacokinetics, by making it less susceptible to enzymatic degradation.

Keywords: Par j 1, allergoid, pharmacokinetics, local immunotherapy, radiolabelling

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Introduction

The local (non-injection) routes for allergen immunotherapy (AIT) represent a matter of considerable scientific interest, especially in European Countries [1]. The overall aim of

non-injection AIT is to minimize the risks for adverse events possibly related to subcutaneous administration, and to provide patients with a more comfortable and acceptable form of AIT. The available literature provides evidence of the efficacy of nasal and sublingual/swallow AIT: both the World Health Organization [2] and the European Academy of Allergology and Clinical Immunology and European Society of Pediatric Allergology and Clinical Immunology

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STUDY DESIGN

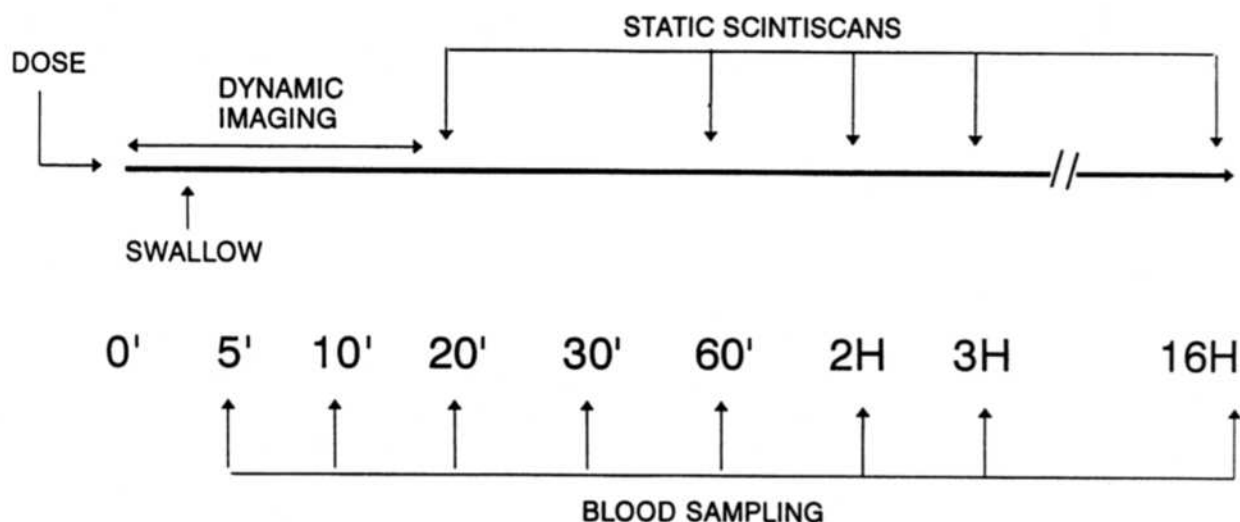


Fig. 1. Study design.

(EAACI/ESPACI) [3] indicate these routes as possible alternatives to injection IT in adults.

In sublingual/swallow AIT the patient is instructed to keep the allergen (prepared as a drop medication or as soluble tablets) under the tongue for 1–2 min, and then to swallow it. This route has been shown to be effective both in improving symptoms and in reducing the need for rescue medications [4–9]. Moreover, it has also been capable of modulating local allergic inflammation [9]. Definition of the pharmacokinetics of an allergenic extract to be employed for AIT is of relevance in order to explore its mechanisms of action and to optimize the administration schedule, especially via local routes. Nevertheless, only sparse data (mostly obtained in animal models) are available on this topic, often providing non-conclusive results [10–12].

In a recent study employing the *Parietaria judaica* radiolabelled major allergen (Par j 1) in healthy volunteers [13], we showed that no direct absorption of the allergen occurs through the sublingual mucosa, while a relevant fraction of the protein persists in the mouth for up to 24 h after administration. Indeed, the swallowed fraction was responsible for most of the radioactivity detected in the blood. However, that study was conducted in healthy volunteers and under experimental conditions, which significantly differ from the administration of the vaccine in clinical use. Therefore, we undertook the present investigation on the kinetics of a commercial preparation of a modified allergen vaccine in allergic volunteers. The

monomeric carbamylated allergoid from the major allergen of *Parietaria judaica* (Par j 1) was radiolabelled with iodine-123 (^{123}I) and incorporated into the commercial orosoluble tablets. The local and the systemic kinetics were then evaluated in allergic volunteers, respectively, by scintigraphic imaging and plasma radioactivity counting, and compared to the kinetics of the native allergen.

Materials and methods

Study design

The present study investigated the absorption and fate of ^{123}I -radiolabelled Par j 1 allergen or allergoid (^{123}I -Par j 1), administered as orosoluble tablets to eight allergic volunteers and to one healthy subject. The kinetics of the radiolabelled allergen in the mouth during and after dissolution of the tablet was evaluated by dynamic scintigraphic imaging. Absorption of the tracer was studied by assessing plasma radioactivity at different time intervals. Gel chromatograms at radioactivity plasma peak were also performed. The experiments were performed between July and December, in order to have all subjects asymptomatic. The overall study design is outlined in Fig. 1.

Subjects

Nine volunteers (five men and four women) aged between 25 and 43 years were enrolled for the study. The routine

laboratory parameters (including free thyroid hormones and thyrotropin plasma levels) assessed prior to the study were within normal range for all subjects. Eight of the subjects suffered from seasonal rhinoconjunctivitis solely due to *Parietaria* pollen, as confirmed by prick test (class ++ or higher) and RAST (class > 1). All the subjects were asymptomatic at the time of the study, they did not take drugs and they had never received AIT before. The ninth volunteer was not atopic.

Thyroidal free radioiodine uptake was minimized by administering a saturated potassium iodide solution (20 drops b.i.d. in the 3 days preceding the study), and potassium perchlorate (200 mg, two capsules on the day of the study). All volunteers signed an informed consent to the study, whose protocol was approved by the Ethical Committee of the Department of Internal Medicine of Genoa University.

¹²³I-radiolabelled allergoid and allergen

Par j 1 allergen was obtained by treating crude *Parietaria judaica* extract with a 90% (NH₄)₂SO₄ solution; the supernatant was then dialysed against 5 mM NH₄HCO₃, and eluted through a high pressure liquid chromatography reverse-phase column (C18, 13 × 300 mm, Deltapack, Waters Italia, Milan, Italy) with acetonitrile/trifluoroacetic acid (0.1%) at the flow rate of 6 mL/min. Fractions containing Par j 1 (as identified with a specific antibody) were pooled, lyophilized and repeatedly chromatographed, to yield a single peak with a molecular weight of 12.5 kDa (SDS-PAGE analysis). The allergen was stored as sterile solution in 0.15 M phosphate-buffered saline at a concentration of about 0.8 mg/mL. The corresponding monomeric allergoid, which constitutes the active principle in the commercial product, was prepared by reacting the allergen with potassium cyanide at alkaline pH in order to selectively carbamylate the ε-aminogroups of the lysine residues [14]. It was stored as lyophilized powder. At variance with the polymeric allergoids (obtained by formaldehyde or glutaraldehyde treatment), the carbamylated allergoids maintain the native molecular size; this characteristic make them suitable for mucosal administration [11].

The allergen (about 30 µg) and the allergoid (about 120 µg) were radiolabelled by the iodogen method [15], using 185–300 MBq (5–8 mCi) of carrier-free ¹²³I (Nycomed-Amersham–Sorin Radiopharmaceuticals, Saluggia, Vercelli, Italy) at the specific activity of 7400 TBq/µM or 200 Ci/µM. Unreacted ¹²³I-iodide (20–50%) was then removed by gel filtration through Sephadex G-25 M pre-packed PD-10 columns (Pharmacia, Uppsala, Sweden). The final radiolabelled proteins had a specific radioactivity of 0.9–1.5 MBq/µg (25–40 µCi/µg). Preliminary

experiments performed with ¹²⁵I-labelling showed remarkable stability to deiodination of the radiolabelled proteins over several days in either physiological saline or serum. ¹²³I was chosen because of its physical properties (main γ emission at 159 keV, half-life 13.2 h). A volume of 50 µL of the tracer (corresponding to 50–100 µCi) was then slowly adsorbed onto the tablets using a micropipette. Each tablet had the same composition as those commercialized for immunotherapy (LAIS, Lofarma S.p.A., Milan, Italy): lactose, microcrystalline cellulose, silicon dioxide and magnesium stearate. Hardness of the tablets ranged between 5 and 7 kg (as determined by durometer), corresponding to a dissolution time of 1–2 min.

Administration procedure

Each subject received a tablet containing the radiolabelled protein as in a normal course of immunotherapy. The tablet had to be dissolved in the mouth and swallowed after its dissolution. Each subject received approximately 1–2 µg of Par j 1 allergen/allergoid per administration, for an approximate radioactivity dose of 1.8–3.7 MBq (50–100 µCi). The tablets were administered within 60 min after radiolabelling. A dynamic scintigraphic acquisition was performed while the tablet dissolved, up to 20 min after administration. Subsequently, the subject was allowed to move, swallow and rinse the mouth.

Scintigraphic acquisitions

A large-field-of-view gamma camera (SP6, Elscint, Haifa, Israel) was used for scintigraphic acquisitions. Early dynamic scintigraphic acquisition upon administration of ¹²³I-Par j 1 consisted of a series of sequential images continuously recorded at the rate of 1 image/10 s while the tablets were dissolving, and for up to 20 min. During such dynamic acquisitions the subject's head was kept close to the gamma-camera collimator. After the initial dynamic imaging single scintigraphic images (static imaging) of the head, chest and abdomen were recorded at 20 min, 1, 2, 3 and, in three cases, 18–24 h after administration of the tracer.

Blood sampling and determination of labelled species

Heparinized 5-mL venous blood samples were collected from each of the allergic subjects during the study. All plasma was separated by centrifugation and 1-mL aliquots were counted for radioactivity content in a well-type gamma-counter. Furthermore, analysis of radioactive species circulating in plasma (samples obtained at 1 and 2 h after administration) was performed by gel-filtration

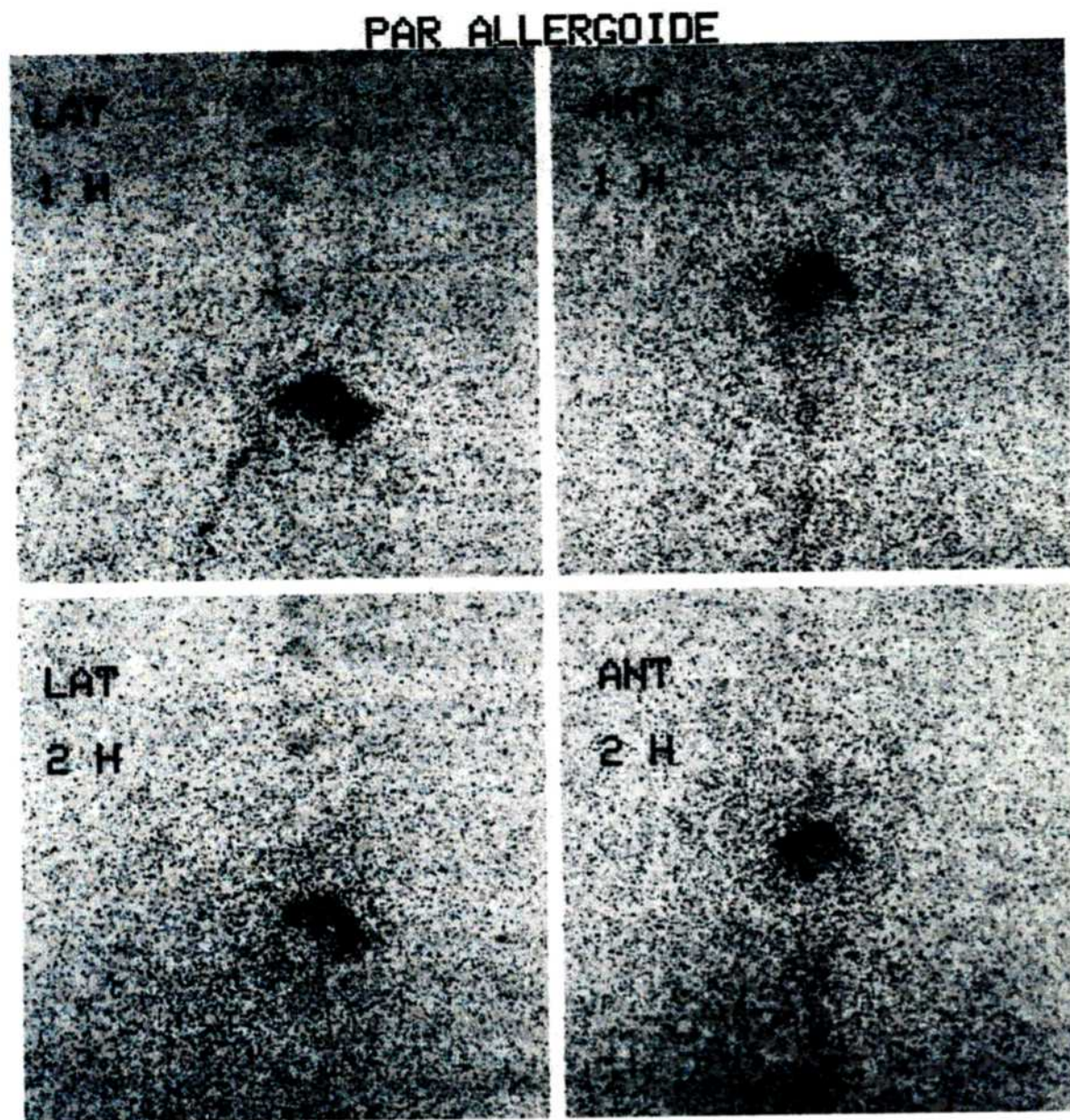


Fig. 2. Static scintigraphic imaging of the head, acquired 1 hour (upper images) and 2 hours (lower images) after the administration of the tablet containing the radiolabelled allergoid. The lateral view (on the left) and the anterior view (on the right) show the persistence of radioactivity in the mouth even after rinsing.

using pre-packed PD-10 columns (Sephadex G-25), as previously described [13].

Results

Comparable specific activities were attained with both the Par j 1 allergen and the allergoid. The chromatographic profile of the radiolabelled allergen and allergoid were closely similar and consistent with the data from our previous study [13]. No adverse event

due to the administration of the radiolabelled proteins was observed. Minimal scintigraphic visualization of the thyroid gland was observed in the three subjects who underwent late scintiscans, despite the thyroid blocking therapy.

Scintigraphic imaging

Dynamic imaging during the early phase (few minutes while tablets were dissolving) showed no change in the

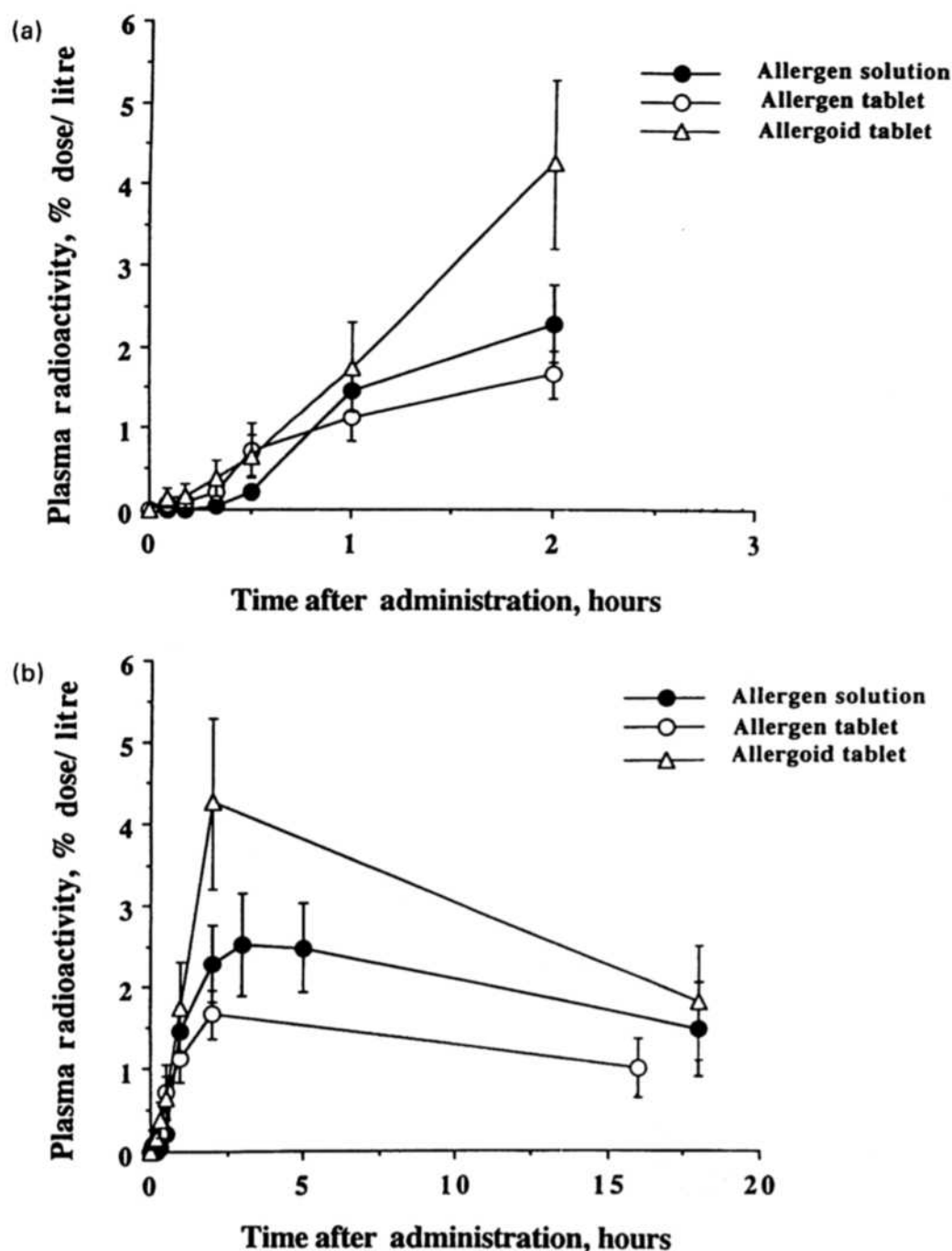


Fig. 3. Plasma radioactivity (% dose/Litre \pm SEM) at different times after the administration of the allergen and allergoid in tablets from 0-time to 3 hours (a) and from 0-time to 18 h (b). The kinetics of the allergen administered as aqueous solution (historical datum [13]), is also plotted for comparison. \bullet = allergen solution; \circ = allergen tablet; Δ = allergoid.

radioactivity content within the mouth. After the tablet had dissolved and the subjects began to swallow, progressive visualization of the pharynx and oesophagus took place as in any oral administration of a radioactive bolus. After swallowing, rapid distribution of the tracer in the gastrointestinal tract took place. A measurable amount of

radiolabelled allergoid (about 2% of the dose) persisted in the mouth for up to 2 h (Fig. 2). The accumulation of the tracer in the mouth at the 3rd hour was almost negligible in all subjects, and no radiolabelled allergoid at all was detectable at late scintiscans. No difference in the local kinetics was observed between the allergen and the

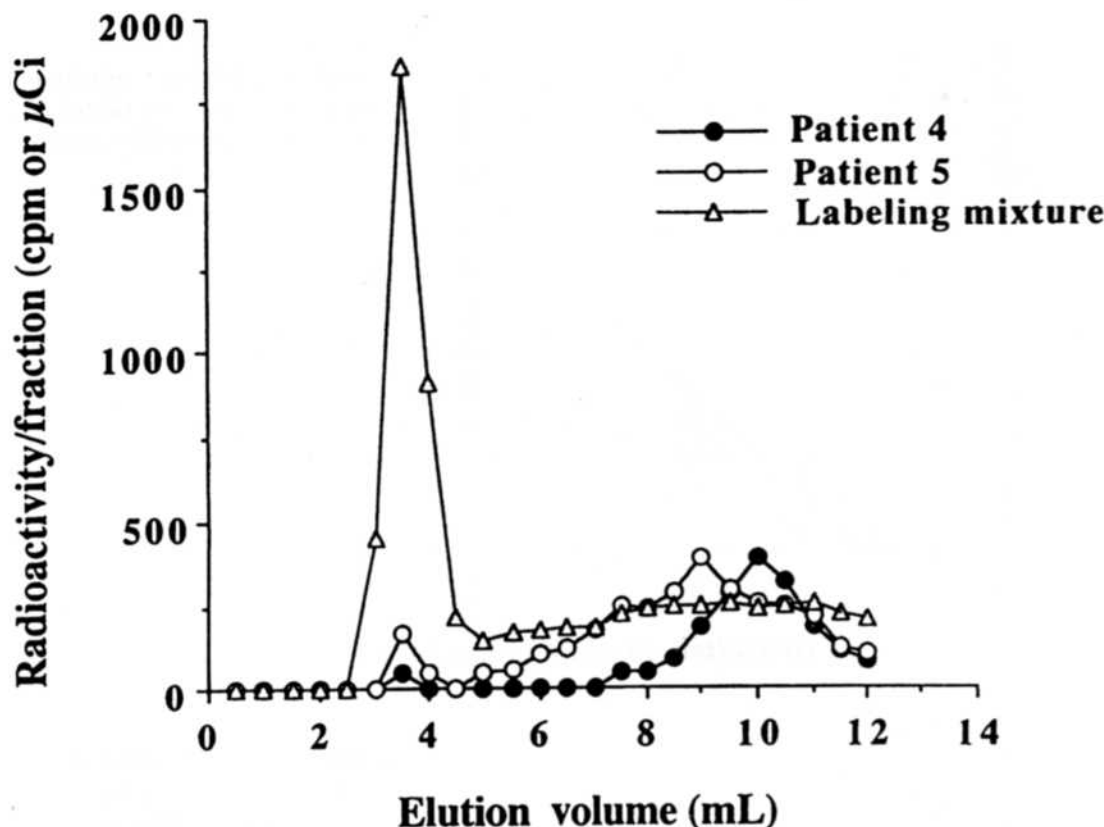


Fig. 4. Gel chromatograms of plasma samples obtained 2 h after the administration of the radiolabelled allergoid to two patients. The chromatographic profile of the allergoid radiolabelling mixture before chromatographic purification is also plotted for comparison. A peak of molecular weight corresponding to the intact allergoid is consistently detectable. ● = patient 4; ○ = patient 5; Δ = labelling mixture.

allergoid and no difference was seen between the allergic volunteers and the healthy subject.

Plasma radioactivity kinetics

The pattern of plasma radioactivity counts over time is plotted in Fig. 3 a, b. Radioactivity was not detectable in plasma until swallowing; after swallowing plasma radioactivity slowly rose and peaked at about 2 hours. Allergen tablet and solution showed a nearly superimposable kinetics. On the other hand, the pattern of plasma radioactivity was different between the allergen and the allergoid: in this latter case, the peak attained was higher ($P < 0.05$, Mann-Whitney test). Gel filtration through Sephadex G-25 of plasma samples obtained at 2 h showed a major peak in the low molecular weight region (consisting mainly of free iodine), as reported in Fig. 4. In patients receiving the allergoid, a small peak corresponding to the molecular weight of the native protein was consistently observed.

Discussion

In a previous study [13] we developed an experimental approach in order to investigate the kinetics of a given radiolabelled allergen and provided preliminary information in healthy humans. In that study the radiolabelled allergen was administered as an aqueous solution, and it was kept under the tongue for 20–30 min without swallowing. These experimental conditions are quite different with respect to the current clinical use. Therefore, we evaluated the kinetics of the commercial vaccine (LAIS Lofarma S.p.A., monomeric allergoid) replicating the conditions of administration to allergic subjects during regular courses of AIT. The radiolabelled allergoid (and the allergen as control), were incorporated into the commercial orosoluble tablets and administered to the subjects following the manufacturer's instructions. Due to the size of the tablet (10 × 3 mm), only a small volume of the tracer could be incorporated, thus implying a low dose of ^{123}I administered and lower radioactivity level detectable. However, good quality scintigraphic images and clearly

detectable radioactivity plasma levels were also observed in these conditions.

Similar specific activities and similar gel filtration radioactivity profiles were attained when labelling either the allergen or the allergoid: in fact, the molecular weights of the two proteins are superimposable (12.5 kDa) and the chemical modification of the allergen (to yield the allergoid) was expected not to affect the efficacy of radiolabelling. It should be pointed out that the kinetics of the allergoid was significantly different from the allergen, since the plasma peak attained was higher. No direct absorption of the radiolabelled allergoid through the mucosa to the bloodstream occurred until the tracer was kept in the mouth: in fact, plasma radioactivity started to appear only after swallowing. Furthermore, a chromatographic peak corresponding to the molecular weight of the allergoid was consistently observed in the 2-h plasma sample; this confirms that part of the protein can be absorbed through the gastrointestinal tract with little or no degradation. This fact may play a relevant role in the mechanisms of action. The increased resistance to gastrointestinal enzymatic degradation can explain the higher absorption of the allergoid and its appearance in the bloodstream. This may be due to the substitution of the majority of $-NH_2$ residues, which are necessary for the hydrolytic action of many enzymes. It is noteworthy that the modification does not affect the capacity of the allergoid to evoke the production of specific antibodies against the non-modified allergen [14].

Some persistence of both the radiolabelled allergen and allergoid in the mouth was observed for up to 2 hours, while in the previous study carried out with the allergen in aqueous solution such persistence was longer; this fact probably depends on the longer time of permanence of the tracer in the mouth. In this study, dissolution of the tablets took place in only about 1–1.5 min (then patients swallowed). Even a short contact of the antigen with the mucosa is sufficient to determine the persistence of the compound in the mouth for hours. In addition, one cannot rule out that longer persistence of part of the administered allergen is not detectable because of the small amount of radioactivity administered. Anyway, this local persistence of the allergens may be consistent with the hypothesis of a possible involvement of mucosal immunity in the mechanisms of action of local AIT.

In summary, the following conclusions can be derived from the present work: first, the main route of absorption (digestive tract) and the persistence in the mouth are similar for the allergoid and the native allergen; second, allergoid vaccine is probably more efficiently absorbed with respect to native allergen.

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