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Allergen Biodistribution in Humans

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Immunotherapy · Radiolabelled allergen · *Parietaria judaica* · Non injective administration ·
Local immunotherapy

Abstract

Specific immunotherapy performed by noninjectable (oral, nasal or oromucosal) routes was mostly developed in the last 20 years with the main aim to avoid side effects that occasionally occur in the course of injectable immunotherapy. Although evidence of its clinical efficacy has been provided some pharmacokinetics aspects are still to be elucidated. In this review we discuss experimental findings of mucosal processing, biodistribution in healthy or allergic humans of ¹²³I-labelled major allergen of *Parietaria judaica* (the most important cause of seasonal allergy in the Mediterranean area) administered by sublingual or nasal routes. The results available to date show that most allergen administered by mucosal route is absorbed via the gastrointestinal tract; however, a proportion is retained at the mucosal level for a relatively long time. These data are potentially useful to improve immunotherapy treatment protocols by noninjectable routes.

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The efficacy of allergen-specific immunotherapy has been largely demonstrated during the last decades, although its action on the immunoregulatory mechanism has been partially elucidated only in recent years [1–7]. The possibility of administering allergen preparations by noninjectable routes has been proposed in the first decades of the 20th century [8, 9]; however, studies concerning noninjectable routes have been developed only over the last 20 years [5, 10–20],

with the main aim being to minimize the possibility of severe side effects, which, although only occasionally, have been observed during the course of allergen-specific immunotherapy through injection [21–23].

An allergen preparation has been administered via direct ingestion (oral route) or by so-called ‘mucosal’ routes: nasal, sublingual or oromucosal. This implies that some local effects or local absorption were expected. As a matter of fact, a demonstration of the clinical efficacy of mucosal allergen immunotherapy has been provided, and some hypotheses about its mechanism of action have been formulated [15–19, 24–32].

However, many points have to be clarified: namely, the mechanism(s) whereby mucosal transit or retention of allergen preparation may exert local or even systemic effects. In fact, these mechanisms are expected to be different depending upon different fate/processing of allergen in contact with different mucosal tissues and their local immune system.

Allergen Handling at the Mucosal Level

Mucosal surfaces are regularly exposed to a wide variety of antigens, including allergens. As a rule, exogenous antigens (and in particular allergens) are expected to be handled by the mucosal immune system in order to clear, at least in part, antigenic challenges before transmucosal absorption and/or to minimize harmful reactions to the organism [33, 34].

As far as specifically allergens are concerned, contact with gastrointestinal or respiratory mucosa appears to deeply modify the systemic response to allergen in experimental animals. In particular IgE response can be reduced or prevented [35–38]. The exact mechanism of such oral ‘tolerance’ is far from clarified; however, the role of antigen processing and presentation, specifically by dendritic cells, is probably critical [24, 33, 39]. Moreover, systemic changes in immunological reactivity to allergen after sublingual treatment were demonstrated in allergic patients [28], although clear evidence of a T cell shift from Th2 to Th1 response is still lacking [32]. In addition some effects on sublingual immunotherapy on adhesion molecule expression and eosinophil activation have been reported [30, 31]. As far as nasal administration is concerned, a number of data from both animal models and human studies suggest an effect on local immunological phenomena.

Specifically, transmucosal absorption has been observed in experimental animals [40–42]; moreover, the mucosal permeability to macromolecules was reported to be higher in allergic than in normal subjects [42, 43]. In allergic patients, intranasal administration of allergen extracts seems to exert its effect mainly on local clinical manifestations and local immunological response [18]. Thus, local administration of allergen may evoke a ‘benign’ systemic immunological

reaction, or, on the other hand, exert its immunological action locally. The former mainly pertains to the sublingual, the second to the intranasal route.

However, although such a perspective has a well-defined theoretical basis and is supported by experimental evidence, no experimental data have been available until the last few years about the fate of allergens administered locally for immunotherapy in humans, and specifically about local retention/persistence, transmucosal passage, and systemic absorption.

Studies with Radiolabelled Allergen in Healthy Humans

Knowledge of the pharmacokinetics of allergen preparation when locally administered is crucial to understand the mechanism of action of local immunotherapy better and eventually to optimize administration protocols.

Data from animal models are scanty and not easily applicable to humans [40–42]. In the late 1990s an experimental approach has been developed to investigate allergen biodistribution in humans [44]: it involves the use of a purified radiolabelled allergen preparation that is administered by different routes to human volunteers. Scintigraphic imaging was used to follow allergen localization and transit as well as analysis of biological fluid radioactivity.

Such studies have been performed with the major allergen of *Parietaria judaica*, Par j 1. *P. judaica* pollen is one of the most widely diffuse allergen in the Mediterranean area. Par j 1 is a glycoprotein of 12.5 kD molecular weight with 2 tyrosine residues; HPLC-purified preparations were used for labelling with radioactive iodine. The radioactive isotope ^{123}I was chosen due to its favorable emission characteristics; its 139-keV gamma radiation peak is optimal for external detection by the γ -camera. The absence of β -radiation emission and the short half-life (13.2 h) allow minimal exposure when administered in vivo with an absorbed total body radiation dose/experiment of approximately 1/30 of a standard chest x-ray film (administered radioactivity 37/56 MBq ^{123}I). For radiolabelling, the iodo-gene method was used, resulting in a radioactive preparation of adequate specific activity (about 3.7 MBq/ μg), with little physical damage of the native molecule and stability to deiodination over several days (as demonstrated in parallel experiments of radiolabelling with ^{125}I , half-life 60 days).

^{123}I -labelled Par j 1 was given to normal volunteers, either sublingually, or orally (direct swallowing of the antigen) or intranasally. The administration schedules and protocols of scintigraphic acquisition and blood sampling are summarized in figure 1. For the sublingual route the dose was delivered in 0.1–0.2 ml under the tongue by a micropipette, then the subject was asked not to move or swallow for 30 min; during this time, a dynamic scintigraphy

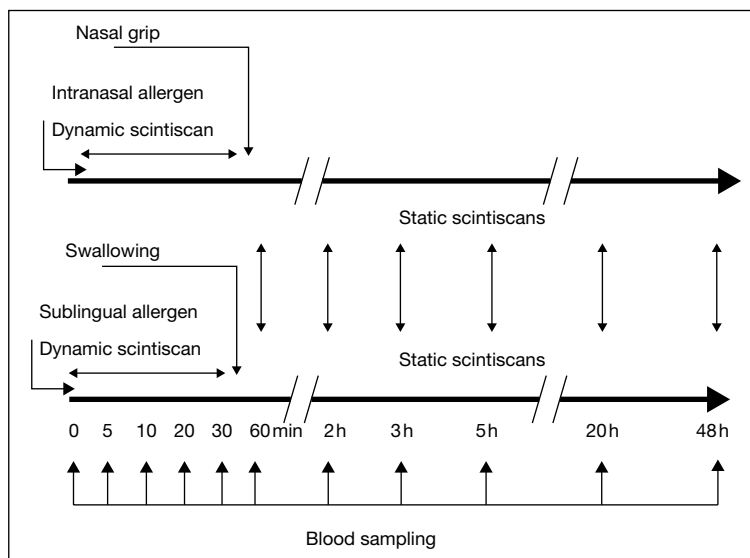


Fig. 1. Study design to investigate allergen pharmacokinetic by ^{123}I Par j 1 administration.

(1 frame/min) was performed of the mouth region. This protocol was chosen to maximize the time of contact with sublingual mucosa.

Other subjects received the allergen sublingually as above, but were asked to spit it into a container provided for this purpose 3 min later; then a new scintigraphic acquisition was started (sublingual-spit protocol) [45]. When the oral route was used, the radiolabelled allergen was administered in 50 ml water and immediately swallowed. For the intranasal route, the dose was sprayed into a nostril by means of a buffer, exactly as for the nasal therapeutic administration, a nasal grip was immediately applied and a scintigraphic acquisition was performed over 30 min. In all cases (see fig. 1) blood samples were drawn for plasma radioactivity counting and plasma chromatography, and static scintigraphic acquisitions were performed at different times (up to 48 h).

In order to minimize thyroid uptake of free radioactive iodine derived from radiolabelled allergen deiodination, saturated potassium iodide solution was given to the subjects in the 5 days before the study followed by potassium perchlorate for 2 days, starting from the day of the study.

Biodistribution of Radiolabelled Allergen by Sublingual Route

Early scintigraphic imaging during sublingual retention of the radioallergen clearly showed no change in the radioactive content of the mouth before

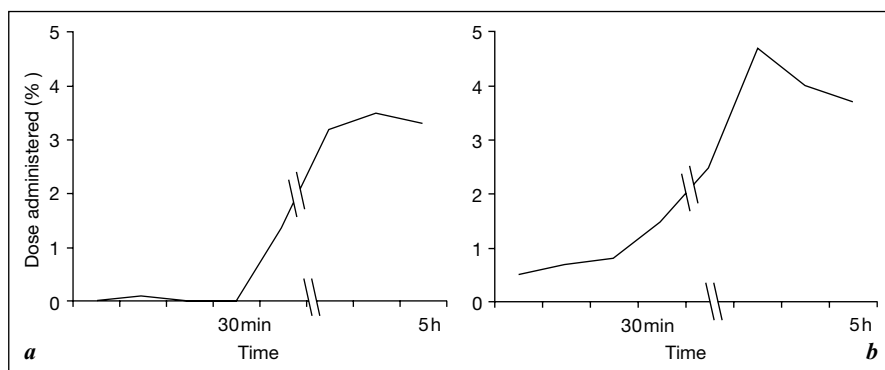


Fig. 2. Plasma radioactivity profiles obtained in 2 healthy volunteers after administration of ^{123}I -Par j 1 by sublingual (a) or intranasal (b) route, respectively. ^{123}I -Par j 1 was administered at time 0; for sublingual administration the subject swallowed at 30 min. The radioactivity is expressed as percent of the administered dose.

swallowing. This was confirmed by the analysis of plasma radioactivity; in fact, plasma radioactivity was at background levels before swallowing and rapidly increased thereafter (see representative experiment of fig. 2).

After swallowing rapid visualization of the esophagus and stomach was obtained, as in all gastrointestinal transit studies with a radioactive bolus. The small intestine appeared a few minutes after the stomach; then renal excretion and visualization of the urinary bladder took place within about 3 h, as shown by static scintigraphic studies. In the control subjects who immediately swallowed the radioactive allergen, rapid distribution of radioactivity in the gastrointestinal tract was apparent, superimposable to what was observed for the sublingual route after swallowing: plasma radioactivity counts, accordingly, strictly paralleled those recorded for the sublingual route after swallowing.

Interestingly, after sublingual, but not oral administration of radiolabelled allergen, a significant amount of radioactivity (about 2% of the administered dose) persisted at the level of the mouth for up to 20 h, even after extensive mouth rinsing, as shown by late scintigraphic acquisition; in control experiments where free ^{123}I was administered, radioactivity disappeared quickly from the sublingual region.

In subjects where the sublingual/spit schedule was applied, radioactivity recovered in the spat saliva accounted for 30% or less of the administered dose; the remaining radioactivity persisted within the mouth, the gastrointestinal tract was visualized, and plasma radioactivity rose immediately after swallowing, as in the sublingual-swallow protocol. This indicates that rapid distribution of the allergen on oral mucosa occurs following sublingual administration and that the spitting procedure does not substantially affect allergen biodistribution [45].

From these data, a direct absorption of allergen from oral mucosa is apparent; the majority of the radiolabelled allergen is swallowed and processed/absorbed in the gastrointestinal tract. It is likely that the systemic immunological effects observed in previous studies are in most part due to gastrointestinal absorption. As mentioned above, even in sublingual-spit protocols the majority of radiolabelled allergen is transported to the gastrointestinal tract. For the same reasons, the risk of too rapid sublingual allergen absorption does not appear to have an experimental basis. On the other hand, it may be hypothesized that retention of a fraction of the administered allergen within the oral mucosa plays a role in the mechanism of action of sublingual immunotherapy, possibly via handling by the local immune system; in fact, processing of administered allergen by dendritic cells of oral mucosa and presentation to T cells have been shown in experimental models [24, 46].

Biodistribution of Radiolabelled Allergen by Intranasal Route

After radiolabelled allergen inhalation, dynamic scintigraphy showed that a remarkable fraction of radioactivity moved from the nose towards the upper pharynx, probably by mucociliary clearance, was gradually swallowed. The esophagus was visualized, followed by the stomach and gastrointestinal tract (as for oral administration); consistently, plasma radioactivity gradually increased over time starting during the first few minutes. No specific radioactivity accumulation was observed at any time at the level of the bronchial tree. It is worth noting, as observed for sublingual administration, that a persistence of radioallergen at the level of nasal mucosa was observed for as long as 40 h. The retained radioactivity was relatively high (about 10% of the administered dose). Also in this case, control experiments with free ^{123}I inhalation did not result in long-term local radioactivity persistence.

As already observed for sublingual administration, even in this case both local retention and systemic absorption of allergen by intestinal tract are likely to occur, the locally retained fraction being higher. Notably the lack of bronchial deposition of radiolabelled Par j 1 seems to minimize the risk of asthma attacks due to undesired bronchial exposure, as previously discussed [5].

Processing of Locally Administered Allergen

Extensive fragmentation of the allergic extracts has been demonstrated to occur in the duodenum [46, 47], whereas some data suggest partial degradation due to saliva (using the RAST inhibition method) [47]. We evaluated the gel

filtration radioactivity profile of saliva after sublingual administration of radiolabelled Par j 1; 0.2 ml of saliva was withdrawn by micropipette from the mouth. The radioactivity profile obtained showed a major peak in the molecular weight region of native allergen, only small fractions of radioactivity being localized in the low molecular weight fractions (fragments, free iodine), as occurs for freshly radiolabelled allergen. Thus, extensive fragmentation of Par j 1 does not seem to occur sublingually [44].

Plasma radioactivity was shown to reach a peak at about 2 h following allergen administration by either the sublingual or intranasal route. Plasma radioactivity gel filtration at 1 and 2 h showed a quite different profile with respect to native allergen, in that only a small fraction of radioactivity was present in the molecular weight region of the intact molecule, whereas the majority was in the low molecular weight region (small peptides and free iodine). This is consistent with extensive deiodination and fragmentation of radiolabelled allergen within the stomach and intestine.

That part of radioactivity in the low molecular weight region which is represented by small molecular fragments, and not only by free iodine, has been demonstrated by ion exchange chromatography; moreover, control individuals assuming free ^{123}I by the sublingual as well as the intranasal route showed a far different plasma radioactivity profile with faster disappearance following the absorption peak. Altogether the available data suggest that the allergen molecule substantially maintains its physical properties during the first mucosal contact, but undergoes extensive degradation within the gastrointestinal tract.

Biodistribution of Radiolabelled Allergen in Allergic Individuals

The above-mentioned results concerning Par j 1 biodistribution were obtained in normal volunteers [44, 45]. In other experiments [48, 49] radiolabelled Par j 1 was administered to volunteers allergic to *P. judaica* by the sublingual or intranasal route. For the sublingual route the preparation was administered in tablets, as in a normal course of immunotherapy; the tablets were kept in the mouth until dissolved, then they were swallowed.

In these experimental conditions too, similar results were obtained; no transmucosal passage of the tracer to the bloodstream before swallowing, as well as local persistence for hours after administration were observed. Accordingly, plasma radioactivity profile and radioactivity gel filtration profile at its peak were similar to the former set of experiments. Preliminary studies were also performed in subjects with allergic rhinitis due to *P. judaica* pollen, receiving radiolabelled Par j 1 intranasally. At variance with what is shown in normal individuals, the disappearance rate of radioactivity from the nasal

region was much faster, and accordingly an earlier peak of plasma radioactivity (1 h) was observed. Moreover, no local long-term persistence of radioactivity at the nasal level was apparent. These data may be consistent with a faster transit of allergen to the upper pharynx and esophagus, but also with local transmucosal absorption.

These data, although preliminary, suggest that local allergic inflammation may affect the biodistribution and fate of the administered allergen, in particular by reducing its local persistence.

Chemical Modification of Allergen: Effect on Biodistribution

Chemically modified allergen (allergoids) are frequently used for immunotherapy. We have comparatively evaluated the biodistribution of native radiolabelled Par j 1 and the corresponding monomeric carbamylated allergoid (monoid) [48].

The results obtained showed no substantial difference in the local kinetics between the two radiolabelled preparations. However, plasma radioactivity profiles showed a significantly higher absorption peak for allergoid: the plasma gel chromatography profile of radioactivity at 2 h constantly showed a well defined, although small, peak in the molecular weight region of intact native allergoid.

Thus, chemical modification of allergen preparation may somehow affect its biodistribution, and part of the protein can be absorbed through the gastrointestinal tract with little or no degradation. An increased resistance of the allergoid to gastrointestinal degradation possibly due to substitution of the majority of NH_2 residues, resulting in reduced sensitivity to enzymatic hydrolysis, could explain the higher absorption of allergoid with respect to allergen.

This fact may have a role in the therapeutic action of the allergoid; it is worth noting that the chemical modification does not affect the ability of the allergoid to elicit a specific antibody response against the native allergen [50].

Conclusions

The studies of allergen pharmacokinetics using ^{125}I -labelled allergen preparation proved to be a unique tool to obtain biodistribution data in humans. The information obtained has provided insight about the mechanism of allergen absorption in humans. Different to what was observed in animal studies [40–42], rapid direct sublingual passage to the blood is not apparent. However, local retention is observed with possible involvement of the local immune system. Nasal mucosal persistence is even more consistent. Anyway, systemic absorption of

extensively processed allergen occurs with both sublingual and intranasal routes, and systemic effects of immunotherapy are probably mainly related to this fact.

Local allergic inflammation may significantly influence the persistence and/or transmucosal absorption of the allergen, and this may have implications for concomitant pharmacological treatments. Finally, chemical modification of allergen may influence its biodistribution. Nevertheless, it should be noted that the short physical half-life of ^{123}I may make it possible to underestimate the local persistence of radiolabelled allergen, in that scintigraphic evaluation is hardly possible some time (more than 48 h) after tracer administration. Moreover, the chromatographic analysis of plasma radioactivity performed to date does not provide information about the chemical nature or biological activity of allergen fragments.

Perspectives

It is important to note that the data available deal with a single purified allergen (Par j 1) and its chemically modified counterpart. The first aim of future allergen biodistribution research will be to investigate other major pollen or perennial allergens with different chemical and physical properties, to draw more general conclusions. In addition, a more detailed analysis of physical and biological properties of peptide fractions resulting from processing of locally administered allergen (as radioactivity HPLC chromatography and/or RAST inhibition studies) on fractions will be required.

All these experimental data, in addition to contributing to a better understanding of the mechanisms of action of local allergen immunotherapy, may make it possible to better devise treatment protocols and allergen/allergoid preparations for therapeutic use.

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