

Pharmacokinetics of allergens after local administration

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The authors review the status of knowledge on absorption and kinetic of allergens administered on nasal mucosa. Intranasal administration is well known in pharmacology, and has been used for a long time for peptide drugs, mainly hormones. The immunological response to intranasally administered antigens have been studied in some experimental models: bovine Rnase, dextran, KLH, tetanus toxoid. These studies showed in atopic people a greater absorption of antigens by nasal mucosa compared to normal individuals, a difference that might be of importance in the pathogenesis of atopic diseases. The absorption rate of peptides through nasal mucosa has been studied biologically by PK-test with Rnase and peanut allergen, and a consistent passage of intact molecules has been observed. Using as tracer ^{125}I -labelled human albumin, transport across the nasal mucosa has been demonstrated in 3/9 normal subjects, 1/9 patients with extrinsic asthma and 9/10 patients with atopic rhinitis. In experimental animals (rabbits) the passage of allergens through the nasal mucosa has been confirmed using ^{125}I -labelled *Parietaria* allergens that showed a peak in the bloodstream 60 min after administration. It is concluded that peptide molecules such as allergens can be absorbed through the nasal mucosa and reach the bloodstream, and that in atopic patients nasal permeability is enhanced.

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The first aspect of intranasal immunotherapy is strictly clinical. Intranasal administration of allergens for hyposensitization purposes came from the observation that repeated allergenic challenges led to local (nasal) hyporesponsiveness (1). Many double-blinded placebo-controlled clinical studies have been performed with such products with encouraging clinical results (this subject has been reviewed by Andri et al., this supplement).

A second aspect concerns the immunological approach. Classical opinion suggests that atopic individuals produce IgE antibodies as a result of overstimulation with antigen and that this occurs through IgA deficiency, which allows the trans-mucosal absorption of abnormal amounts of antigen. Experimental work with laboratory animals indicates a mechanism that is the antithesis of the overstimulation concept, that quantities of antigen sufficiently large to activate IgE are normally absorbed across the mucosae and that in normal animals suppression, rather than IgE, responses result (2). More precisely, atopic animals, i.e. high-

IgE-responders, require a larger quantity of antigen (in the range 100–1000 fold) to reach IgE-restricted immunological suppression compared to normal animals, i.e. low-IgE-responders (3). In the present paper we will examine the pharmacological and immunological aspects of the intranasal administration route. More precisely, the experimental demonstrations of absorption of antigenic molecules through nasal mucosa will be reviewed.

Pharmacology of nasal administration of drugs

For systemic medication, drugs are routinely administered by parenteral (i.v., i.m., s.c.) or oral routes. In some cases, where systemic medication is required but the parenteral administration is impractical (e.g. daily consumption), the oral route seems to be the only choice. Nevertheless, in many cases oral administration may not be suitable because of bioavailability problems associated with gastrointestinal degradation and hepatic first-pass

metabolism. This is the case with peptide drugs, which are particularly subject to enzymatic proteolytic degradation. In view of vascularity underlining the nasal mucosa, the possibility of by-passing hepato-gastrointestinal first-pass elimination and ease of administration, the nasal route seems to be the ideal alternative for frequent administration of some drugs.

In recent years it has been demonstrated that many drugs are able to achieve better systemic bioavailability by medication through the nasal route. The main classes of drugs which have been evaluated for intranasal administration are:

- Cardiovascular drugs (e.g. nitroglycerine as vasodilator for angina pectoris, propofanol, adrenergic β -receptor blocking drugs);
- Immunologically active drugs (e.g. interferon, influenza virus vaccine);
- Hormones (e.g. growth hormone, luteinizing hormone releasing hormone (LHRH), oxytocin, vasopressin, calcitonin, insulin);
- Autonomic drugs (e.g. ephedrin, atropine, ipratropium);
- CNS stimulants (cocaine HCl); and
- Prostaglandins.

The nasal absorption of drugs have been studied by *in-situ* nasal perfusion experiments (4). The trachea of anaesthetized rats was cannulated and a second tube, which served to introduce the perfusion solution into the nasal cavity, was inserted through the oesophagus to the posterior part of the nasal cavity. The nasopalatine was sealed with an adhesive agent to prevent drainage of the drug solution from the nasal cavity into the mouth.

From these experiments it was found that nasal absorption of peptides is lower than that observed for other drugs, and that it is not strictly related to the size of the peptide (5). It is believed that the nasal bioavailability of peptides can be influenced by polarity (high polarity seems to decrease absorption rate).

A different experimental approach is direct evaluation of the pharmacological effect of the drug, e.g. the induction of ovulation in rats treated with LHRH and its analogues. These pharmacological studies led to the conclusion that the nasal administration of many drugs with the advantage of rapid absorption, similar to subcutaneous injection and ease of administration, is an important option when long-term treatment with peptides is required. The bioavailability of peptide drugs after nasal administration ranges between 1% and 20%, depending upon molecular size, physicochemical properties, enzyme resistance and other factors. In Table 1, from Evans et al. (6), some examples of

Table 1. Peptides administered by intranasal route, absorption rate and molecular characteristics (modified from Evans et al. (6))

Peptide	Absorption %	No. amino acids
TRH	20	3
Vasopressin analogues	6-12	9
LHRH, agonists/antagonists	1-10	9-10
ACTH analogues	12	17-18
Growth hormone releasing factor	1-2	40-44

the relationship between nasal bioavailability and molecular size are reported. With regard to the formulation, nasal spray administered with squeeze bottles or metered-dose nebulizers was the most easy solution, and it has been adopted for the first clinical studies with allergens. Nevertheless, it is well known that allergenic extracts are poorly stable in aqueous solution, being subject to enzymatic degradation during storage and to absorption phenomena on the inner surfaces of the containers (7). For these reasons a new formulation has been developed, consisting of allergens incorporated into inert powder excipient (lactose). These powder allergen formulations showed extraordinary stability, even when stored at room temperature (up to 6 years when measured with RAST-inhibition assay; unpublished data), and have been used successfully for many double-blinded placebo-controlled clinical studies (see Andri et al., this supplement).

Immunological responses to intranasally administered antigens

Some work has been undertaken to ascertain if there is a difference in the immunological responses between atopic patients and normal individuals. In the first study, by Leskowitz & Lowell (8), the immunological response to dextran and pneumococcal polysaccharide has been studied following subcutaneous injection. No differences between the groups (normal and atopic) have been observed in respect to precipitine antibodies and skin reactivity. In addition, no differences in skin reactivity could be shown for the pharmacological agents histamine, serotonin and the histamine releaser compound 48/80. It was then suggested that the essential difference between atopic and normal individuals lay in the method by which allergens coming into contact with mucosal surfaces was absorbed and handled. The subsequent study by Salvaggio et al. (9), using bovine ribonuclease (RNase) as antigen, was undertaken to confirm this suggestion. The antigen (240 μ g of crystalline RNase) was administered by intranasal inhalation dispensed in a DeVilbiss nebulizer once per week for 3 months. Intense immediate wheal

and erythema reactivity was observed by intradermal skin testing at the end of the treatment course in all 10 atopic individuals studied, while the 10 control normal individuals were negative. The results of this study were confirmed by further experiments using aerosolized dextran (10) and keyhole limpet haemocyanin (11) as antigens.

Interesting information about the immunological response to intranasal antigens have come from the studies performed with tetanus toxoid (12, 13). Such antigen had been administered in groups of atopic (positive to common inhalant allergens) and normal individuals, by droplets placed into the nostrils, and immunization was boosted after 15 days. Serum and nasal secretion samples were collected before and after immunization and used to determine the content of immunoglobulins and specific anti-tetanus toxoid antibody. Non-atopic subjects demonstrated higher IgA-antitetanus toxoid levels in nasal secretions and in serum after intranasal immunization. Such findings may provide a clue in defining a protective role for secretory IgA antibodies in atopy. Conversely, atopic individuals demonstrated higher nasal IgE antitetanus toxoid antibodies. Also, the serum concentration of IgE antibody was higher in atopic subjects. A significant rise in IgA and IgG nasal wash antitetanus toxoid antibody levels was, however, observed in both groups throughout the course of intranasal immunization.

On the basis of these studies it became clear that, while all individuals possess the capability to produce IgE antibodies, among atopic people there is a greater absorption of antigens through the nasal mucous membranes compared to normal individuals, a difference that might be of importance in the pathogenesis of atopic diseases caused by inhalant allergens. Nevertheless, these types of study cannot offer physical demonstration of permeability to macromolecules in atopic individuals, but are limited to the evaluation of immunological responses.

Nasal mucosal absorption of allergens

Studies in humans

Studies to assess mucosal absorption more directly have been performed by determining the capacity of allergens applied intranasally to elicit cutaneous Prausnitz-Kuestner (P-K) reactions in normal individuals and atopic subjects sensitive to other allergens (14). Two series of reaginic human serum dilutions were injected intracutaneously into recipients' backs: one series consisted of sera allergic to RNase (experimentally sensitized volunteers), the other series consisted of sera of patients allergic to peanuts.

Forty-eight hours later one series of intradermal sensitization sites was challenged intracutaneously

with the first allergen under assay (RNase), and the results of the skin tests were recorded. After 30 min the second allergenic extract under assay (peanuts extract) was then sprayed into the nose, and the second series of skin results was recorded. Both the allergens under assay elicited positive P-K tests both after intracutaneous and after intranasal administration. It was a clear demonstration that allergens were absorbed and reached systemic circulation. The atopic group failed to show greater or more rapid absorption of either allergen through the nose. Nevertheless, the main goal of this study, i.e. to demonstrate the capacity of antigenic molecules to pass through intact nasal mucosa keeping their biological (allergenic) activity, was reached.

An important milestone in the studies of nasal mucosal permeability to macromolecules in humans was the study of Buckle & Cohen (15), in which absorption of albumin labelled with ¹²⁵Iodine through the nasal mucosa was compared in atopic and normal subjects. This study was also designed to test the hypothesis that patients with atopic rhinitis and extrinsic asthma have a nasal mucous membrane defect that allows inhaled macromolecules to be absorbed and to have access to immunocompetent cells. Three groups of patients were studied: normal subjects, patients with extrinsic asthma and patients with atopic rhinitis. Labelled albumin (¹²⁵Iodine, 20 µCi) was applied to the nasal mucosa and blood samples were drawn at different intervals up to 4 h. The blood samples were submitted to dialysis, gel-filtration and immunoprecipitation procedures to discriminate between free and albumin-bound radioactivity. Significantly larger amounts of albumin-bound radioactivity were detected in the plasma of patients with atopic rhinitis compared with that of normal subjects after 32 min ($P < 0.001$). 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 ($P < 0.02$). This study did not determine whether the increase in nasal transmucosal transport observed in such patients is a cause or an effect of atopic rhinitis, but showed that intact large molecules are able to pass across the nasal mucous membrane of patients. These experimental data are suggestive for the intranasal administration of allergens, because albumin has a molecular size much larger than the allergens of clinical interest from pollens and mites.

Studies in experimental animals

The pharmacokinetic of allergens has been studied by Mistrello et al. (16) with the following experimental model:

- 1) Allergenic extract of *Parietaria judaica* was instilled into the nostrils of normal rabbits.
- 2) Blood samples were taken at different time intervals, and the blood allergenic concentration determined by immunocapture-RAST. This was a new and sensitive assay consisting in the coating of polystyrene beads with an affinity-purified anti-*Parietaria* antibody, in the incubation with serum samples, in subsequent reaction of "captured" allergens with IgE antibodies from human allergic sera and then with ^{125}I -anti-IgE. This assay has a sensitivity in the order of nanograms per ml.
- 3) In parallel, control rabbits were treated via intravenous route with the same extract.

In three different experiments, a consistent absorption of allergens was observed in the rabbits' blood, up to 100 ng/ml. The allergen was detectable in serum after 30 min, reached a peak at 60 min and decreased after 120 min. As expected, the comparison of kinetic profiles obtained after intravenous and intranasal administration showed that at the time of maximum absorption by the nasal route (60 min) the allergenic activity was lower, about 10% of that detectable at the same time after intravenous injection. This is not surprising considering that absorption through nasal mucosa can be influenced by molecular size (*Parietaria* allergens range from 10 kDa to 80 kDa). On the basis of these studies it is appropriate to stress two aspects:

- 1) The studies have been performed in normal healthy animals. The above-mentioned studies conducted in normal volunteers and atopic patients have shown clearly that atopic individuals have an enhanced mucosal permeability. Therefore it is presumed that the observed absorption rate could increase in sensitized animals.
- 2) Other studies have been made with allergens labelled with radioactive tracers. In the present case a crude not-treated allergenic extract was used. Also, in the mentioned study the immunological activity of the allergen which reached the bloodstream was detected, being such detection assay (immunocapture-RAST) based on recognition by both rabbit *Parietaria*-specific IgG antibodies and by IgE from human allergic sera. Therefore the bioavailability of the allergen after nasal administration is unquestionable.

In other experiments rabbits have been treated intranasally with birch pollen extract, and their immunological response compared to animals treated parenterally (i.v. and s.c.). It was evident that by the intranasal route the immunological

response was restricted to the heat-stable components of the pollen extract which are related to the allergenicity in human patients, while parenterally treated animals developed immunological response also against nonallergenic heat-labile pollen components (17). Antibodies in the nasal secretions appeared to be directed mainly against the heat-stable allergenic components, and therefore it is reasonable that these antibodies may be acting as blocking antibodies.

Conclusions

- 1) Intranasal administration route of peptides is valid and adopted for many drugs. Also antigens and allergens can be delivered through this route.
- 2) In the case of antigens (allergens) the adsorption rate is obviously lower compared to parenteral route, but the amounts of antigen absorbed are sufficient to elicit immunological responses.
- 3) Patients with atopic rhinitis have a higher nasal permeability to allergens compared to normal subjects.
- 4) Bioavailability of antigens after nasal absorption has been demonstrated.
- 5) After nasal administration allergens appear in the blood-stream with a peak at 60 min (demonstrated for *Parietaria*).

In conclusion, the intranasal administration route of allergens has been accepted both to drive the antigenic stimulus to the nasal-associated lymphoid tissue (NALT) and for a systemic absorption. Therefore, there is a rationale for its use in local immunotherapy.

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