

Short communication

Permeation of polysucrose 15 000 across the human nasal mucosa *in vivo*

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Studies of the nasal permeation of small molecules (<1000 Da) have yielded important information about the integrity of the human airway mucosa in health and disease. In this study, we used a much larger tracer molecule, polysucrose (PS) 15 000 (approx. 14 700 Da), to predict the mucosal permeation of inhalational allergens. PS 15 000 (50 mg/ml; 15 ml), with or without a detergent type of permeation enhancer (dioctyl sodium sulfosuccinate 10 mg/ml), was maintained for 15 min in one nasal cavity of 12 healthy nonatopic subjects by employment of a nasal-pool device. Permeation as determined by the 24-h urine recovery of PS (micro-ELISA analysis assay) was expressed as percentage of nasal instillate. Mean baseline permeation was 0.044% (range 0.009–0.250%). In the presence of the detergent, permeation increased to 0.600% (range 0.007–2.260%) ($P < 0.01$). After oral intake of 750 mg of PS 15 000 (50 µg/ml; 15 ml), the 24-h urinary recovery was 0.013% (range 0.004–0.023%). Our study thus demonstrates a measurable baseline permeation of PS 15 000, an elevated permeation rate in the presence of an epithelium-damaging detergent molecule, and a negligible permeation by the oral route. These properties support the utility of PS 15 000 as a nasal airway permeation tracer. Its size further suggests that its permeation may reflect mucosal perviousness to many allergens.

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The nose is a main port of entry for environmental noxious agents. Hence, the nasal mucosa is exposed to allergens, infectious agents, and pollutants, which may be harmful unless they are neutralized by nasal mucosal defense systems. A major defense factor is the tightness of the epithelial lining, which prevents penetration and permeation of deposited noxi. Mucociliary transport, together with nose-blowing and sneezes, further minimizes the dwelling time of the foreign material on the airway surface. Recent observations on exudation mechanisms, epithelial repair processes, and the function of the mucosal barriers are thus the basis of a new *in vivo* paradigm of airway tightness in allergy (1–5). Although increased perviousness to inhaled molecules can no longer be regarded as a general characteristic of allergic airways, permeation of allergen-sized molecules remains an important determinant of how much allergen reaches antigen-

presenting cells and other immunocompetent cells in the mucosa.

Previous studies of nasal permeation of small molecules (<1000 Da) have generated important information about the integrity of human airway mucosa in health and disease (2, 6, 7). In contrast, large molecules, such as radiolabeled albumin, have failed as permeation tracers in the human nose (8), the failures being due to rapid gastrointestinal permeation of swallowed tracer and/or too low nasal permeation rates (8), which prevented proper analysis.

PS 15 000 is a fairly large carbohydrate with a molecular weight of about 15 000 Da and a molecular diameter of 4.2 nm (9). Hence, the weight (and size) of PS 15 000 may be of the same magnitude as that of commonly inhaled pollens and major allergens in pollens (10, 11). PS 15 000 has previously been used for permeation studies in the

human gastrointestinal tract (12) where the baseline permeation rate is low. To control for several variables that may affect the nasal permeation, we used a nasal-pool device in the present study (13).

This study aimed to explore the utility of PS 15 000 as a nasal permeation tracer.

Material and methods

Subjects

Twelve healthy, nonatopic subjects, eight men and four women, participated. Their mean age was 24.5 years. They all had a previous negative skin prick test to common allergens, and none had a history of chronic nasal or chest disease. Physical examination of the upper airways revealed no abnormality in any subject. All subjects had previously participated in nasal experiments and were well versed in the techniques used in the present study. All subjects gave informed consent, and the study was approved by the local ethics committee at Lund University, Sweden, and conducted according to the Declaration of Helsinki.

Study design

The study consisted of three challenges and a washout period of 1 week between each challenge. The nasal permeation test was performed with PS 15 000 (50 mg/ml) dissolved in 15 ml of isotonic saline. (Preliminary experiments employing 10 mg/ml of PS 15 000 resulted in too low permeation rates across the human nasal mucosa; unpublished observations.) At the second visit, PS 15 000 was given in the same concentration combined with a detergent (see below). At the final appointment, the same total amount of PS 15 000, 750 mg, was given *per os*. In order to avoid large volumes of urine, all patients were instructed not to drink alcoholic beverages 2 days before and during the urine collection. All permeation tests were performed at the same time of day, and a pretest urine sample was collected to serve as baseline control. All urine was collected after the exposure, the final portion of urine being obtained at 24 h on all three occasions.

Nasal permeation measurement

The instillation fluid was 15 ml of solution. The fluid was instilled in the unilateral nasal cavity with a compressible plastic container (13). The adapter was inserted into the nasal opening while the subject sat in a forward flexed neck position (60° from the upright position). The pool device was filled with PS 15 000 (50 mg/ml) dissolved in

isotonic saline, thus giving a total amount of 750 mg of the tracer in each pool device. Approximately 12 ml of fluid was introduced into the unilateral nasal cavity, but the rest volume of roughly 3 ml in the device was still in contact with the part instilled in the nasal cavity. As the subject was leaning forward, swallowing of the fluid instilled was minimized. A trickle of fluid could, however, drain through the contralateral nasal cavity, but this occurred only during the first seconds of the instillation procedure and only in a few subjects. (These subjects did not exhibit any aberrant permeation behavior.) The instillate was kept in the nasal cavity for 15 min, after which the pressure was released and the fluid returned to the container. Two additional lavages with isotonic saline were then immediately performed to rinse the surface of any remaining tracer. The 24-h urine volume was measured, and, in one aliquot, the level of PS 15 000 was determined by micro-ELISA (9). The amount of the excreted PS 15 000 was calculated and expressed as percentage of the nasally administered dose.

The detergent, dioctyl sodium sulfosuccinate (DOSS), was dissolved in ethanol and added to the instillate. The concentration of DOSS in the instillate was 10 mg/ml, and the concentration of ethanol was 50 mg/ml. Even lower doses (6 mg/ml of detergent and 30 mg/ml of ethanol) increase the permeation of small hydrophilic molecules about six times, as previously observed (6).

PS 15 000 preparation

PS polymers with a broad molecular weight range were prepared by cross-linking PS with epichlorohydrin in alkaline medium (14). The molecular weight distribution of different PS fractions was determined by size-exclusion chromatography (SEC) on a calibrated Superose 12 HR 10/30 column (Pharmacia LKB, Uppsala, Sweden). The calibration was achieved by chromatography (15), and the Stokes radius was determined (16). The PS preparation used for the present study, PS 15 000, had a mean molecular weight of 14 700 Da, corresponding to a Stokes radius of about 2 nm.

PS 15 000 measurement

The assay procedure for measuring PS 15 000 in urine samples has been described in detail earlier (9). In brief, one 2.5-ml urine aliquot from each urine sample was gel filtered, to remove low-molecular-weight components, and the macromolecular fraction of the sample was analyzed by an inhibition immunoassay in duplicate. The samples were incubated in a microtiter plate coated

with rabbit anti-PS antibodies, and then a mixture of streptavidin-alkaline phosphatase and biotin-PS was added and coincubated. The plate was washed and developed, and the concentration of PS determined against a standard curve. Intra- and inter-assay variations were 7% and 11%, respectively.

The excreted proportion of PS 15 000 in relation to intake was calculated by the following formula:

$$\frac{\text{Conc} \times \text{Vol} \times 100}{\text{Dose}} = \text{Excr\%}$$

Conc = concentration of PS 15 000 in 24-h urine sample (ng/ml)

Vol = urine volume (ml)

Dose = total administered dose of PS 15 000 (7.5×10^8 ng)

Excr% = excretion in percentage of administered dose.

Statistics

Statistical analysis of paired observations was performed by the Wilcoxon signed rank test. *P* values <0.05 were considered significant.

Results

All patients completed the study. There were no detectable levels of PS 15 000 in the urine samples collected immediately before the first nasal instillation, and no differences were found in the urine volumes collected at the different test occasions: 1440 ml (range: 650–2450) after nasal instillation of tracer; 1570 ml (range: 680–2800) after nasal instillation of tracer and detergent; and 1450 ml (range: 820–2700) after oral intake.

The fraction of PS 15 000 excreted in 24-h urine in the control situation ranged from 0.009% to 0.250% (mean 0.044%) of the nasal instillate. In the presence of the detergent, the nasal permeation of PS 15 000 was remarkably increased to 0.600% (mean) of the instillate (range: 0.007–2.260%) ($P < 0.01$ compared to the first visit). The amount of PS 15 000 in 24-h urine after the entire pool fluid had been swallowed was 0.013% of the instillate (range 0.004–0.023%). The individual values of excreted PS 15 000 after nasal instillation of PS 15 000 with and without the detergent are shown in Fig. 1.

Discussion

The present study demonstrates several aspects of the nasal permeation of PS 15 000 in nonatopic, healthy human subjects. For example, with a defined permeation time of only 15 min, there is a significant baseline permeation of this tracer molecule.

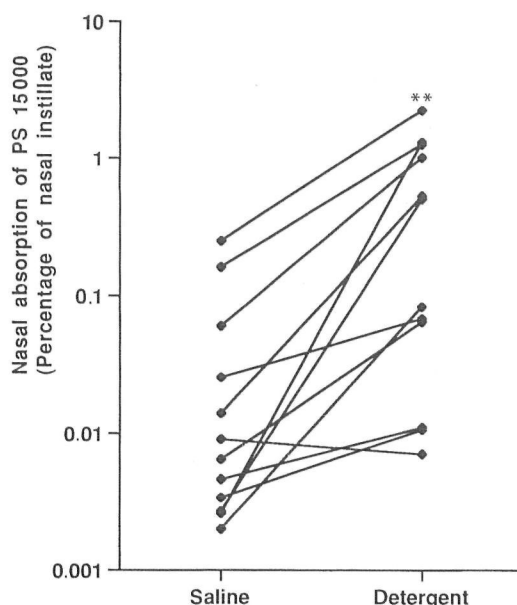


Fig. 1. Excreted polysucrose 15 000 (in percentage of nasally instilled dose) in 24-h urine sample with and without detergent in 12 healthy subjects (log values). Rate of permeation was significantly increased after addition of detergent to nasal instillate (** $P < 0.01$).

The present degree and variation of baseline permeation rates, as well as the increased permeation induced in this study by an acute epithelium-damaging detergent, are of interest, since PS 15 000 may behave like commonly inhaled allergens.

PS 15 000 is 13–40 times larger than Desmopressin and ^{51}Cr -EDTA, which have previously been employed as permeation tracers to elucidate the integrity and barrier function of the human nasal mucosa in health and disease (2, 5, 6, 13). These three tracers are hydrophilic molecules that, irrespective of size, probably use paracellular epithelial routes when penetrating the airway mucosa. Although permeation routes still need to be examined in detail (4), the postulated pathway for intestinal permeation of PS 15 000 and ^{51}Cr -EDTA is also between epithelial cells (9, 18). Both these tracers are thus orally absorbed, but only to a low extent, and both have been used to study mucosal conditions in inflammatory bowel disease (12, 17). A low degree of oral permeation is mandatory for nasal permeation tracers because nasal-to-gut displacement of tracer cannot be completely avoided. The undesired contribution of oral permeation is minimized by the present pool technique (13) and also by the low degree of oral permeation of PS 15 000 (5, 8). The present experiments involving oral intake of the entire nasal-pool tracer solution thus demonstrated an exceedingly low oral permeation of PS 15 000 (amounting to about 0.01% of the given dose).

It is remarkable that the baseline permeation of PS 15 000 across the nasal mucosa during 15 min is three to four times greater than that occurring along the entire gastrointestinal tract during a time period that theoretically could amount to 24 h. While this intriguing behavior of PS 15 000 remains unexplained, it is considered a very important asset for its utility as a nasal permeation tracer. Its nose-to-gut permeation ratio potentially allows the use of other application techniques, such as nasal sprays, which involve a greater risk of gut displacement than the present nasal-pool technique.

The present small group of subjects, all being young nonatopic adults in good health and with apparently normal nasal airways, exhibited a 25-fold difference in the rate of permeation of PS 15 000. This observation suggests that the healthy nasal mucosa may absorb allergen-sized molecules at highly varying rates. Indeed, if surface-removal mechanisms are efficient, many subjects may not appreciably absorb this size of allergen molecules. It would be of interest to determine the nasal mucosal capacity to absorb PS 15 000 in atopic subjects. Studies in seasonal allergic rhinitis outside and inside the disease period would be of particular help to clarify abnormalities in the mucosal handling of molecules of this size. Such information is now urgently needed to complement current studies on the role of environmental allergen load in the development of allergic airway disease. Furthermore, information on permeation may be needed to clarify the interaction between allergens and the subepithelial effector cells.

In the acute presence of DOSS, permeation of PS 15 000 was increased about 13-fold. This result tallies with the effect of DOSS, given acutely at the same concentration as in this study, on nasal permeation of a small tracer molecule (6). The mechanism behind the permeation enhancement probably involves the acute epithelial damage (4, 18) that is associated with a plasma exudation response (19).

We conclude that PS 15 000 is an interesting addition to the available permeation tracers. It is not radioactive, and it is the size of allergen molecules. We suggest that PS 15 000 may be particularly useful in studies of the function of the human airway mucosa as a barrier to inhaled and locally deposited allergens.

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