

The “Nasal Pool”-device for challenge and lavage of the nasal mucosa in children: Histamine-induced plasma exudation responses

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The accessibility of the nasal airway allows important examination of the airway mucosa in health and disease. However, the current methods for nasal challenge and lavage in children suffer from several shortcomings. In the present study, we have assessed the utility of a recently developed “nasal pool”-device in 7–9 year old children, and explored the ability of the nasal mucosa of these school-children to mount a plasma exudation response (luminal entry of bulk plasma). Isotonic saline was instilled and maintained (1 min) as a “pool” in the unilateral nasal cavity. Recovery of the “pool” (lavage fluids) was determined. Concomitant challenge and lavage was then performed by exposing the nasal mucosa to a “pool” of isotonic saline and histamine (40–400 µg/ml) for 2 min. “Pool” fluids were analysed for α_2 -macroglobulin as an index of microvascular-epithelial exudation of bulk plasma. The school-children successfully managed to carry out nasal lavages as well as concomitant histamine challenges and lavages with the “nasal pool”-device. The recovery of the nasal lavage fluids was almost quantitative (>85%) and thus well reproducible. Histamine produced significant exudation of bulk plasma (α_2 -macroglobulin). We suggest that the “nasal pool”-device is well suited for challenge and lavage of the nasal airway mucosa in children above 6 years of age, and conclude that luminal entry of multipotent humoral protein-systems may be an important first line respiratory defence mechanism in children.

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In human nasal airways, in health and disease, challenge and lavage techniques have proved to be very useful tools in studies of soluble microvascular and cellular indices of inflammation (1). Different techniques to obtain nasal mucosal surface liquids in children have been reported: Absorption by filter-paper (2) and suction by catheters (3), saline lavages using syringes (4), instillations in the “lateral decubitus position” (5, 6), and administration of saline with metered dose nasal inhalers (7). However, these techniques may show inconsistencies in relation to baseline data and reproducibility, and their characteristics in children have been little described (1–7). Where recovery of the lavage fluids has been reported it has been less than 50% (7). The previously employed techniques may also have limitations due to a required co-operation from the young subjects.

Thus the devices are generally handled by the investigator which, occasionally, may be regarded as an infringement upon the integrity of the child.

We have introduced the “nasal pool” technique for challenge and lavage of the nasal airway mucosa in adults (8). The “nasal pool”-device is a compressible plastic container with a nasal adapter. When compressed, by the subject sitting in a 60° flexed neck position, the unilateral nasal cavity is filled with fluid. With this atraumatic technique a large area of the nasal mucosal surface can be gently exposed during defined periods of time (up to 15–20 min in adults). Challenge- and tracer-agents can be added to the lavage fluids allowing concomitant mucosal exposure and lavage almost in organ-bath like conditions. It is our experience that the nasal pool-technique requires little training and co-operation.

Hence, this technique may be envisaged to be suitable also for challenge and lavage of the child's nasal airway.

In the present study, we have examined the possibility of using the "nasal pool" device for challenge and lavage of the upper airways in 7–9 year old children. In addition we have explored the occurrence of a plasma exudation response in these children. Exudation of bulk plasma (microvascular-epithelial exudation) has previously been proposed as a major first line defence mechanism of the airway mucosa in adults (9). In this study, we have thus examined effects of nasal challenges with increasing concentrations of an exudative mediator, histamine. Levels α_2 -macroglobulin in the lavage fluids have been determined and used as an index of luminal entry of bulk plasma. The challenge and lavage fluids have been instilled and maintained in the uni-lateral nasal cavity using the "nasal pool"-technique.

Material and methods

Study design

The first part of the study examined the possibility that school-children themselves can perform lavages of the nasal mucosa using the "nasal pool"-device. The recovery of the lavage fluids were monitored. Furthermore, the lavage fluid levels of α_2 -macroglobulin were measured as an index that the lavage fluids had been in contact with the nasal mucosa. The second part of the study, examined if histamine produces mucosal exudation of bulk plasma (α_2 -macroglobulin) in the upper airway in school-children. The study was of an open design.

Subjects

In the first part of the study, 22 children (aged 9 years, 14 boys) participated. Nine of these children and two additional children (aged 7–9 years, seven boys) participated in the second part of the study. The children had no history of chronic nasal disease and no history of recent common cold, as assessed by a verbal parental questionnaire. The study was approved by the local ethics committee and informed consent was obtained from the children and their parents.

Nasal pool-device

The nasal pool-technique can be used for nasal lavage as well as concomitant nasal challenge and lavage. It is a compressible plastic container equipped with a nasal adapter (Fig. 1). The adapter is inserted into one of the nostrils and the container is com-

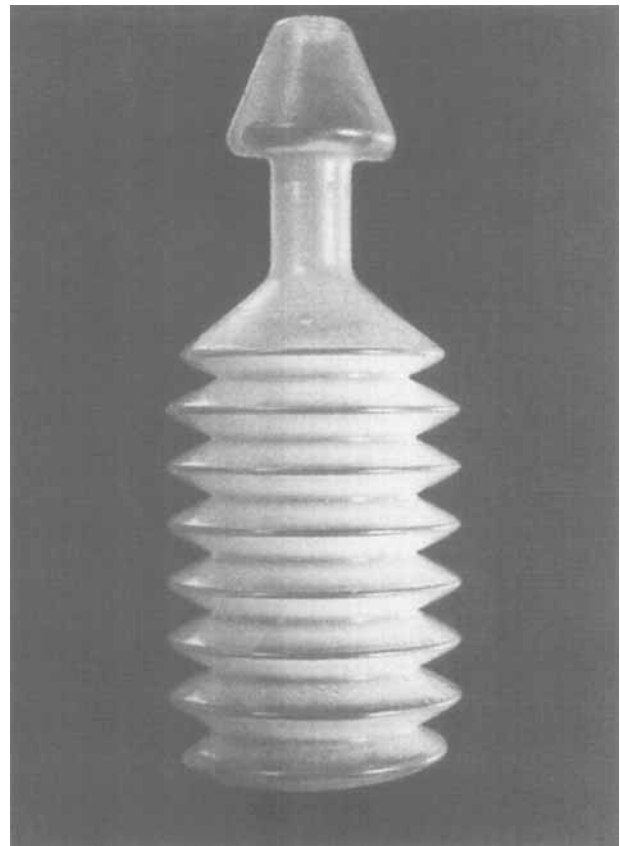


Fig. 1. Photograph of a "nasal pool"-device. The actual height of the device is about 8 cm.

pressed by the subject while sitting leaning forward in a 60° flexed neck position. The nasal pool-fluid is then instilled in one of the nasal cavities and maintained in contact with a large and defined area of the mucosal surface for an extended period of time. When the pressure on the device is released, the fluid returns into the container.

Isotonic saline lavage of the nasal mucosa

In this the first part of the study, a single 1 min lavage with isotonic saline was carried out. The nasal pool-device contained 10 ml fluid. The recovery of the lavage fluid was monitored (calculated as the recovery of the initial nasal pool-device weight) and the lavage fluid level of α_2 -macroglobulin was analysed. The recovered lavage fluid was centrifuged ($G=105$ g, 10 min, 4°C) and aliquots prepared from the supernatants were frozen (–20°C) for later analysis of α_2 -macroglobulin. The children were also instructed to anonymously summarise their experience of the nasal lavage procedure in writing.

Histamine challenge and lavage of the nasal mucosa

In this the second part of the study (Fig. 2), an initial 1 min "pool" lavage with isotonic saline was carried out. Then, three 2 min concomitant challenges and lavages with isotonic saline, histamine (40 µg/ml), and histamine (400 µg/ml) were carried out at 7 min intervals. Furthermore, 1 min lavages with isotonic saline were carried out 5 min after each of the concomitant challenge and lavages. The nasal pool-devices each contained 10 ml fluid. The recovered lavage fluids were prepared as described above. The lavage fluid concentrations of α_2 -macroglobulin were measured as an index of mucosal exudation of plasma and the recovery of the lavage fluids were monitored. The intra-individual reproducibility of the recovery between the first and the third 1 min lavage was calculated. Nasal blockage was scored (score 0: none, 1: mild, 2: moderate, and 3: severe blockage) and number of sneezes counted after each 2 min concomitant challenge and lavage.

Analysis of α_2 -macroglobulin

The lavage fluids were put in coded vials and α_2 -macroglobulin were measured using a radio immunoassay sensitive to 7.8 ng/ml. Rabbit anti-human α_2 -macroglobulin (Dakopatts, Copenhagen, Denmark) was used as anti-serum and standard human serum (Behringwerke Diagnostica, Marburg, Germany) as standard. Human α_2 -macroglobulin (Cappel-Organon Teknika, Turnhout, Belgium) was iodinated using the lactoperoxidase method. Tracer and

standard (or sample) were mixed with anti-serum before adding goat anti-rabbit anti-serum (Astra Draco, Lund, Sweden). The bound fraction was measured using a gamma counter (Pharmacia, Uppsala, Sweden). The intra- and inter-assay coefficients of variation were between 3.8–6.0% and 3.1–7.2%, respectively.

Statistics

Differences in volume recovery and α_2 -macroglobulin concentrations in the second part of the study were first analysed by the Friedman test and if a statistical significance emerged by the Wilcoxon signed rank test. A p-value less than 0.05 was considered significant. Data are presented as mean $\pm$ s.e. mean.

Results

The children managed to carry out nasal lavages as well as concomitant histamine challenges and lavages after a single demonstration of the technique but without any practical training. All children described the lavage procedure in positive terms and none reported any degree of discomfort (as assessed by the childrens' written summary in the first part of the study).

In the first part of the study, 20 of the 22 1 min nasal lavages were carried out correctly as indicated by a quantitative recovery of the lavage fluids ($93 \pm 1.0\%$) together with expected lavage fluid levels of α_2 -macroglobulin (0.52 ± 0.14 µg/ml) (10). Two initial experiments failed since the lavage fluid recovery was 100% and in these lavage fluids there were no detectable α_2 -macroglobulin.

In the second part of the study (Fig. 2), all lavages appeared to have been carried out correctly. In the four one minute lavages, the recoveries were $85.0 \pm 2.2\%$, $92.3 \pm 2.9\%$, $90.0 \pm 2.2\%$, and $88.9 \pm 2.0\%$. The mean of the intra-individual coefficients of variation between the recovery of the first and the third one minute lavages (i.e., lavages not affected by any histamine-induced mucosal swelling) was 8.9%.

Histamine produced mucosal exudation of plasma (α_2 -macroglobulin) as detected in the concomitant challenge and lavage fluids as well as in the lavage fluids obtained 5 min after the concomitant challenge and lavage (Fig. 3A and B). The recovery of nasal lavage fluids after the concomitant challenge and lavage was for isotonic saline, histamine (40 µg/ml), and histamine (400 µg/ml), respectively, $91.8 \pm 1.7\%$, $84.5 \pm 2.1\%$, and $88.6 \pm 1.8\%$. Histamine produced a moderate nasal blockage and few sneezes: The score of blockage was 0, 0.1 ± 0.3 , and 0.5 ± 0.5 for isotonic saline, histamine (40 µg/ml),

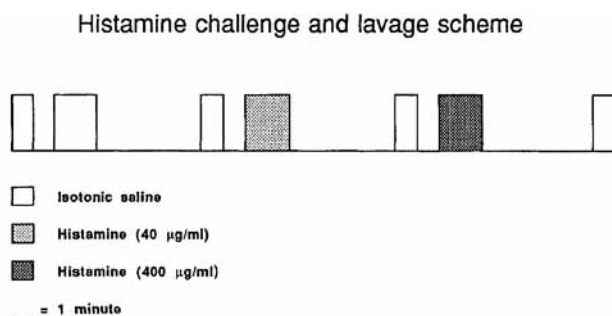


Fig. 2. Histamine challenge and lavage scheme (i.e., the second part of the study): Initially, a 1 min isotonic saline lavage was carried out to remove mucosal surface liquids that might have accumulated on the mucosa. Thereafter, isotonic saline and histamine (40 and 400 µg/ml) in isotonic saline was instilled. These instillations were carried out with about 7 min intervals and each instillate was kept in the nasal cavity for 2 min (see Fig. 3A for results). Furthermore, a 1 min isotonic saline lavage was carried out 5 min after each 2 min instillation (see Fig. 3B for results).

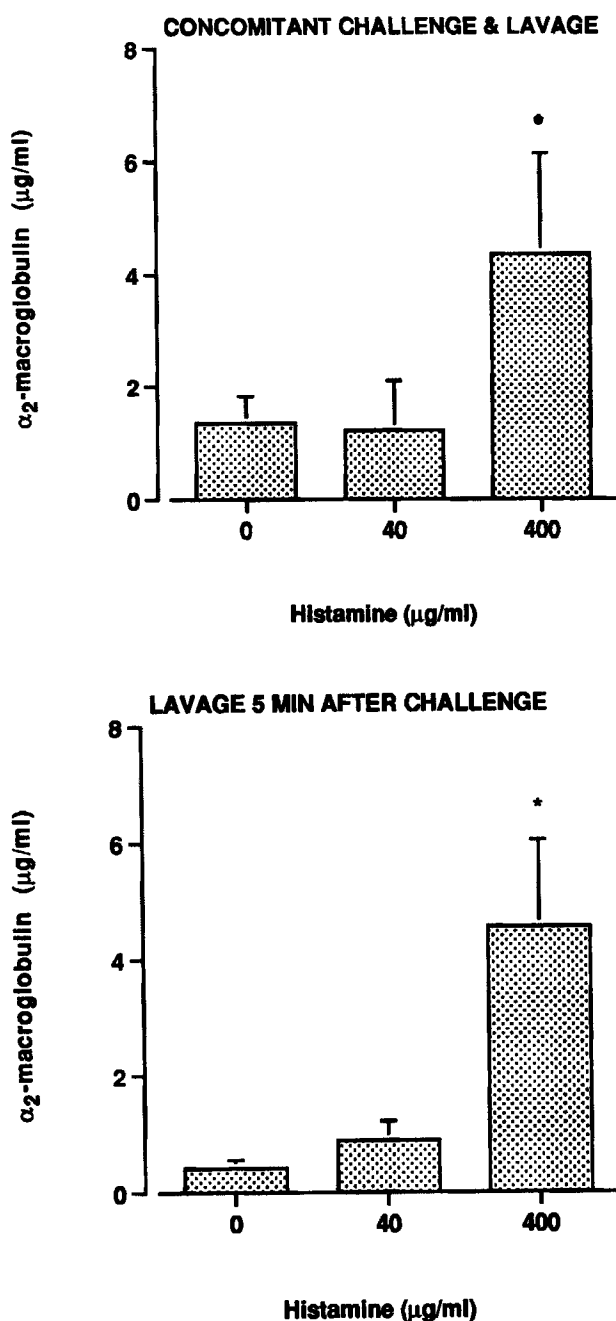


Fig. 3. Histamine produced mucosal exudation of plasma (α_2 -macroglobulin). This response was evident in the concomitant challenge and lavage fluids (A) as well as in the lavage fluids obtained 5 min after each challenge (B). (* $p < 0.05$.)

and histamine (400 $\mu\text{g/ml}$), respectively, and the number of sneezes were 0, 0, and 0.5 ± 0.8 .

Discussion

This study demonstrates some of the useful properties of the "nasal pool"-device for examination of

the nasal mucosa in 7–9 year old children. We have further demonstrated that topical histamine evokes acute exudation of the large plasma protein α_2 -macroglobulin across the nasal mucosa in these children. The present method may be an important tool in studies of the physiology and pathophysiology of the airway mucosa, including the exudation response, in children.

The school-children successfully managed to perform challenges and lavages with the "nasal pool"-device and they needed only brief instructions to accomplish this. The volume of the "nasal pool"-fluid was 10 ml and, without drainage of excess fluid through the contra-lateral nasal passage, about 70% of this volume was instilled into the uni-lateral nasal cavity by compressing the device. The recovery of the nasal lavage fluids in the non-blown nose was quantitative ($>85\%$) and reproducible, in agreement with previous findings in adults (8). The degree of recovery was far greater than, for example, that of less than 50% reported recently by Noah et al. (7), who employed a metered dose inhaler to administer 7 ml of saline to a large group of children aged 7–12 years. The present results indicate that the "nasal pool"-device can be successfully employed in studies of the upper airway in school-children. Hence, the properties of this technique for combined challenge and lavage can thus be utilised in children: A large area of the nasal mucosa can be reached with defined concentrations of challenge agents, and the same mucosal surface can be lavaged concomitantly during a selected period of time. These properties may not be achieved by previously employed techniques (1–7). As an additional asset of the "nasal pool"-technique, and potentially of special value for this age group of test subjects, the present challenge and lavage procedures were performed by the school-children themselves after minimal training. Whether the "nasal pool"-device can be employed in pre-school-children now remains to be examined.

Histamine challenge-induced mucosal exudation of plasma was evident already during the brief exposure (2 min) to active histamine levels and it was well established in the lavages that were obtained 5 min after the histamine challenge. These data agree with the notion that in children as well as in adults histamine-induced movement of bulk plasma from the subepithelial microcirculation into the lamina propria and across the intact epithelial lining occurs rapidly. The exudative response to a single dose of histamine may continue to develop during at least 10 min (8). Histamine-induced symptoms may also develop more fully when the mucosa is exposed for longer time periods than employed in the present study. An increased contact-time between the challenge and lavage fluids and the mucosa, which is feasible in adults (8), would further reduce the varia-

bility in the response to histamine challenge. However, shorter time periods, such as those employed in the present study, were found to be suited for the use of "nasal pool" challenge and lavage in school-children. In these test subjects it is thus recommended to carry out repeated lavage to pick up a full response rather than maintain lavage fluids in the nasal cavity for prolonged times.

In studies involving adults, we have previously demonstrated that histamine produces parallel nasal mucosal exudation (luminal entry) of albumin, fibrinogen, and α_2 -macroglobulin (10). It is therefore likely that the increase in lavage fluid levels of α_2 -macroglobulin (mol wt. 720 kDa) demonstrated after histamine-challenge in the present study reflects mucosal exudation of bulk plasma. By its rich content of biologically active molecules mucosal exudation of plasma has been considered a major airway defence mechanism on the mucosal surface (9). Despite its non-sieved nature luminal entry of plasma is a neatly regulated non-injurious process: After extravasation from the subepithelial microcirculation there is a transient phase when the lamina propria is flooded with bulk plasma. In less than a minute the plasma exudate moves up between and all around the epithelial lining cells (9, 11). Possibly through a hydraulic mechanism the exudate makes ubiquitous para-cellular pathways into the airway lumen. The epithelial crossing of bulk plasma is non-injurious as it leaves the structure of the epithelium intact (9, 11). Furthermore, the function of the mucosa as an absorption barrier appears to be uncompromised by this process: In studies involving guinea-pig tracheal and human nasal airways we have demonstrated that inflammatory mediators, including histamine, produce marked mucosal exudation of plasma whereas the absorption ability is unaffected (9, 12–14). The present demonstration that a simple mediator such as histamine, that would not in any way damage the epithelial lining, caused significant luminal entry of plasma is strong evidence suggesting that the normal airway mucosa of 7–9 year old children has a well developed mechanism for microvascular-epithelial crossings of all the powerful protein systems of circulating blood. Hence, we propose that mucosal exudation of plasma is a major first line respiratory defence mechanism in these children.

Mucosal exudation of plasma may also reflect the intensity and the time course of epithelial-subepithelial inflammatory processes in allergy and infection (15, 16). As further examined by histamine challenges in adults, the exudative responsiveness of the subepithelial microcirculation may be increased in airway inflammation (10, 17). Using the present technique it would thus be possible to examine both baseline exudation and exudative responsiveness in airway disease conditions in children, such as aller-

gy and infection. For several reasons it is difficult to examine mechanisms of plasma exudation and mucosal absorption in the human tracheobronchial airways with accuracy:

- (1) The concentration and distribution of challenge agents and lavage fluids on the airway mucosa cannot be well controlled.
- (2) It may be difficult to distinguish between lavages of the airway and the alveolar surface.
- (3) The baseline data vary due to uncontrolled factors including the time factor and the involved mucosal surface area.

It is therefore of interest that the upper and the lower airways exhibit similar plasma exudation responses to inflammatory challenges: e.g., in adults, histamine challenge produces mucosal exudation of plasma in nasal (10) and bronchial (18) airways. In agreement with previous suggestions (19), the present findings in the nasal airway may be valid also for the bronchial airways in school-children. This potential relevance is of particular importance in children where challenge and lavage of the tracheobronchial mucosa for research purposes is not widely accepted.

We conclude that the "nasal pool"-device is suitable for challenge and lavage of the nasal airway in school-children. Hence, important *in vivo* characteristics of the nasal mucosa in health and disease may be well examined in children by this technique. Based on the present data, we suggest that luminal entry of multipotent plasma peptide- and protein-systems is a major defence mechanism in children.

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