Effects of experimental changes in nasal airway pressure on mucosal output of plasma.

Berg, Sören; Wollmer, Per; Andersson, Morgan; Persson, Carl; Greiff, Lennart

Published in:
Clinical Physiology and Functional Imaging

DOI:
10.1046/j.1475-097X.2003.00487.x

2003

Citation for published version (APA):
Effects of experimental changes in nasal airway pressure on mucosal output of plasma

Søren Berg1, Per Wollmer2, Morgan Andersson1, Carl G. A. Persson3 and Lennart Greiff1

1Department of Otorhinolaryngology, University Hospital, Lund, Sweden, 2Department of Clinical Physiology, University Hospital, Malmö, Sweden, and 3Department of Clinical Pharmacology, University Hospital, Lund, Sweden

Correspondence
Lennart Greiff, Department of Otorhinolaryngology, University Hospital, SE-221 85 Lund, Sweden
Tel.: +46-46-171705
Fax: +46-46-2110968
E-mail: lennart.greiff@skane.se; lennartgreiff@hotmail.com

Accepted for publication
Received 26 September 2002; accepted 21 January 2003

Key words
asthma; inflammation; mucosa; nasal; rhinitis

Summary
Microvascular extravasation, lamina propria flooding and luminal entry of plasma are key features of airway inflammation. We have suggested that the extravasated plasma moves across the epithelial lining along hydrostatic pressure gradients. The present study, involving healthy subjects, tests this hypothesis by examining effects of experimentally applied negative and positive luminal pressures on nasal output of plasma at baseline and at histamine-induced plasma exudation. The negative (−10 cmH2O) and positive (10 cmH2O) pressures were applied for 10 min after nasal spray administrations of diluent (saline) and histamine (0·5 mg). The mucosa was then lavaged and the lavage fluid levels of α2-macroglobulin were measured as index of plasma exudation. Nasal administrations of diluent and histamine (0·5 mg) were also carried out without any pressure applications. Histamine produced significant mucosal exudation of plasma. The negative luminal pressure augmented this response significantly as well as the baseline appearance of α2-macroglobulin in mucosal surface liquids. We conclude that extravasated plasma may be moved across the epithelium by a hydrostatic pressure-operated epithelial mechanism.

Introduction
Microvascular extravasation, lamina propria flooding and luminal entry of bulk plasma are key features of airway inflammation (Persson et al., 1998). Accordingly, topical airway applications of various inflammatory mediators, including histamine, produce increased levels of different-sized plasma proteins in airway mucosal surface liquids (Greiff et al., 2002). Also, this response has been well documented in airways disease characterized by inflammation, such as allergic rhinitis (e.g. Naclerio et al., 1983) and asthma (e.g. van der Graaf et al., 1991). The most important implication may be that adhesive, leucocyte-activating, growth-factor active, complement-active, and otherwise biologically active plasma proteins will promptly operate in and on an insulted but still intact airway mucosa (Persson et al., 1998).

Plasma extravasation is induced by actions of inflammatory mediators on endothelial cells of subepithelial, postcapillary venules (Majno & Palade, 1961; Majno et al., 1961): These cells are separated and plasma is extravasated into the lamina propria. We have demonstrated that the plasma exudate rapidly enters the airway lumen, and we have suggested that plasma also moves across the epithelial lining aided by a pressure-operated mechanism, allowing epithelial cells to separate transiently and plasma to move into the airway lumen via a paracellular routes (Erjefält & Persson, 1989; Persson et al., 1998). This hypothesis is supported by observations in vitro demonstrating that application of a slight hydrostatic pressure (5 cmH2O) on the serosal aspect of guinea-pig tracheal tube preparations readily produces luminal entry of macromolecules (Persson et al., 1990; Gustafsson & Persson, 1991).

In the present study, involving healthy subjects, we have tested the above hypothesis by examining whether or not experimentally applied negative and positive nasal airway luminal pressures affect the nasal mucosal output of plasma at baseline and at histamine-induced plasma exudation. We have thus employed a lavage technique and we have monitored the lavage fluid levels of the plasma protein α2-macroglobulin (mol. wt 720 kDa) as index of luminal entry of bulk plasma.

Material and methods

Subjects
Fifteen healthy subjects (aged 22–34 years, mean age 26 years) participated in the study. The subjects had no history of chronic or recent nasal disease and no history of ongoing or recent drug treatment. The study was approved by the local research ethics committee and informed consent was obtained.
Changes in nasal airway pressure and mucosal output of plasma, S. Berg et al.

Study protocol

The subjects were examined on four occasions:
(i) Diluent challenge (saline) without subsequent negative nasal pressure challenge.
(ii) Histamine (0.5 mg) challenge without subsequent negative nasal pressure challenge.
(iii) Diluent challenge with subsequent negative nasal pressure challenge (~10 cmH2O).
(iv) Histamine (0.5 mg) challenge with subsequent negative nasal pressure challenge (~10 cmH2O).

Ten of these subjects were also examined on two additional occasions:
(i) Diluent challenge with subsequent positive nasal pressure challenge (10 cmH2O).
(ii) Histamine (0.5 mg) challenge with subsequent positive nasal pressure challenge (10 cmH2O).

Nasal challenges with either isotonic saline or histamine (50 mg/ml) were administered by means of an adapter (Greiff, Marburg, Germany) as standard. Human α2-macroglobulin (Cappel-Organon, Turnhout, Belgium) was iodinated using the lactoperoxidase method. Tracer and standard (or sample) were mixed with antiserum before adding goat antirabbit antiserum (AstraZeneca, Lund, Sweden). The bound fraction was measured using a gamma counter (Pharmacia, Uppsala, Sweden). The intra- and inter-assay coefficients of variation are between 3.8–6.0 and 3.1–7.2%, respectively.

Statistics

Wilcoxon signed rank test was used to examine differences in lavage fluid concentrations of α2-macroglobulin. A P-value less than 0.05 was considered significant. Data are presented as mean ± SEM.

Results

The baseline appearance of α2-macroglobulin was low and consistent with previously reported baseline levels (Persson et al., 1998; Greiff et al., 2002). Furthermore, histamine produced an expected, marked mucosal output of α2-macroglobulin (P<0.001).

The baseline appearance of α2-macroglobulin (P<0.01) as well as the histamine-induced mucosal output of α2-macroglobulin (P<0.05) was increased by the application of a negative luminal pressure (~10 cmH2O) (Fig. 1).

The application of a positive nasal pressure (10 cmH2O) reduced the baseline appearance of α2-macroglobulin as well as the histamine-induced plasma exudation to some extent, but these changes failed to reach statistical significance (Fig. 2).

Figure 1. α2-Macroglobulin in nasal lavage fluids obtained at baseline (saline challenge) and following challenge with histamine in the absence and presence of a negative pressure challenge. Histamine produced a marked plasma exudation response (significance levels are given elsewhere). The negative pressure increased this response as well as the baseline appearance of α2-macroglobulin (*denote P<0.05 and **denote P<0.01).
In the subgroup of subjects that underwent negative as well as positive pressure challenges (n = 10), the negative pressure challenge was associated with greater lavage fluid levels of \( \alpha_2 \)-macroglobulin after the diluent challenge (P<0.05) as well as after histamine challenge (P<0.05) compared with the positive pressure challenge.

**Discussion**

The present study has demonstrated that nasal mucosal output of plasma, as indicated by lavage fluid levels of \( \alpha_2 \)-macroglobulin, is sensitive to experimental changes in the nasal airway (luminal) pressure. This finding is in agreement with our hypothesis that airway luminal entry of extravasated plasma involves a hydrostatic pressure-operated, valve-like epithelial mechanism.

After the extravasation of plasma from the subepithelial microcirculation, there is a phase when the surrounding lamina propria is flooded with plasma proteins. Promptly, the plasma exudate then moves up between and around the epithelial lining cells, and makes ubiquitous paracellular pathways into the airway lumen (Erjefält et al., 1995). Although this process involves movement of bulk plasma, including the largest plasma proteins such as \( \alpha_2 \)-macroglobulin (this study), the luminal entry may not damage the epithelial lining (Erjefält et al., 1995), nor does it increase mucosal absorption, i.e. the perviousness of the epithelium to luminal solutes (Greiff et al., 1991a,b; Persson et al., 1998). We have previously suggested that this unidirectional increase in the outward permeability of the epithelial lining reflects a hydrostatic pressure-operated valve-like mechanism whereby a slight increase in the subepithelial hydrostatic pressure, created by the plasma exudate itself and its attracted fluids, allows epithelial cells to transiently separate. In the present study, the observation that luminal entry of plasma proteins, at baseline and at exudative conditions, is affected by hydrostatic pressure changes supports our hypothesis that luminal entry of plasma proteins occurs along hydrostatic pressure gradients.

\( \alpha_2 \)-Macroglobulin represents a specific binding capacity of plasma proteins (Peterson & Venge, 1987). The present data on luminal entry of bulk plasma (\( \alpha_2 \)-macroglobulin) is in agreement with the possibility that extravasated plasma may bind various pro-inflammatory factors, e.g. eosinophil cationic protein (ECP), occurring in the tissue, and move them to the airway surface for clearance through mucociliary transport and other mechanisms. Rinsing mucosal interstices, including the para-epithelial spaces, may be a component of the innate immunity role of plasma exudation. However, the most important implication may be that adhesive, leucocyte-activating, growth-factor active, complement active, or otherwise biologically active plasma proteins will promptly operate not only in the mucosal tissue but also on the surface of an insulted but still intact airway mucosa.

We conclude that experimental application of a negative luminal airway pressure augments baseline appearance of plasma proteins in nasal mucosal surface liquids as well as histamine-induced mucosal exudation of bulk plasma. This finding supports the view that extravasated plasma may move across the epithelium along hydrostatic pressure gradients.

**Acknowledgment**

We thank Mrs Lena Glanz-Larsson for technical assistance.

**References**


