

# Topical nitroprusside may reduce histamine-induced plasma exudation in human nasal airways

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Mucosal exudation of nonsieved bulk plasma is a key feature of airway defense and inflammation. We have previously observed in guinea pig tracheobronchial airways that endogenous nitric oxide (NO) of the mucosa may tonically suppress the permeability of the subepithelial microcirculation, and that topical administration of the NO donor nitroprusside may reduce plasma exudation responses. The present study examines whether nitroprusside affects histamine-induced mucosal exudation of plasma in the human nasal airway. In a dose-finding tolerability experiment, using changes in nasal patency as response, placebo and nitroprusside (1.2 and 3.6 mg per nasal cavity) were applied on the mucosal surface with a nasal-spray device. Nasal peak expiratory flow (PEF) rates were measured before the application and thereafter every third minute for 15 min. Nitroprusside produced a dose-dependent decrease in nasal PEF rates compared to placebo. Placebo or nitroprusside (7.2 mg) was then given to the right nasal cavity, followed 3 min later by challenge with saline or histamine (600 µg). The drug and the challenge were both applied with a nasal-spray device. With a nasal pool-device, the same large part of the nasal mucosal surface was lavaged before and after the treatment/challenge. The lavage fluid levels of  $\alpha_2$ -macroglobulin were measured as an index of mucosal exudation of bulk plasma. The histamine-induced lavage fluid level of  $\alpha_2$ -macroglobulin was significantly higher after treatment with placebo than with nitroprusside. The present data indicate that nitroprusside may have antiexudative effects in human airways. Hence, unlike other microvascular permeability active agents, this pharmacologic principle may be active in both guinea pig and human airways.

L. Greiff<sup>1</sup>, M. Andersson<sup>1</sup>, C. Svensson<sup>1</sup>, M. Nilsson<sup>2</sup>, I. Erjefält<sup>2</sup>, J. S. Erjefält<sup>3</sup>, C. G. A. Persson<sup>4</sup>

Departments of <sup>1</sup> Otorhinolaryngology, Head and Neck Surgery and <sup>4</sup> Clinical Pharmacology, Lund University Hospital, Lund; <sup>2</sup> Department of Pharmacology, Astra-Draco, Lund, <sup>3</sup> Department of Medical Cell Research, Lund University, Lund, Sweden

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Lennart Greiff  
Department of Otorhinolaryngology, Head and Neck Surgery  
Lund University Hospital  
S-221 85 Lund  
Sweden

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Mucosal exudation of bulk plasma is a key feature of airway defense and inflammation (1, 2). Accordingly, increased lavage fluid levels of plasma proteins of various size, e.g., albumin (69 kDa) and  $\alpha_2$ -macroglobulin (720 kDa), have been shown in rhinitis and asthma (3, 4). The mechanisms of this response involve active separation of endothelial cells of postcapillary venules followed by extravasation and luminal entry of nonsieved plasma (mucosal exudation) (5). The epithelial passage may occur via hydrostatic pressure-operated paracellular gates (1, 5). Thus, luminal plasma, which can readily be sampled by lavage procedures, reflects the extent of increased macromolecular permeability of the subepithelial microcirculation, which in turn may reflect

the intensity of the mucosal inflammatory process (5).

We have recently shown that topical airway administration of *N*-nitro-L-arginine methyl ester (L-NAME), which acts as a false substrate for nitric oxide (NO) synthase and therefore blocks NO formation, produces mucosal exudation of plasma across the guinea pig tracheobronchial mucosa (6). Furthermore, we have shown that removal of the epithelium *in vivo*, without damage to the basement membrane, is a potent stimulus of plasma exudation (7). These findings suggest that one action of NO produced by the airway mucosa may be to tonically suppress the macromolecular permeability of the subepithelial microcirculation. In extra-airway tis-

sues of animals, NO has been reported either to increase (8) or decrease (9, 10) the microvascular macromolecular permeability. In guinea pig tracheo-bronchial airways, topical administration of the NO donor nitroprusside reduces an acute mediator-induced plasma exudation response (11). NO synthase activity may be present in both guinea pig and human airway epithelium (12, unpublished observation by Erjefält JS, et al.). Whether or not NO or NO donors affect the permeability of the human microcirculation seems an important question in view of the fact that several pharmacologic principles, including neurogenic agents (13, 14), glucocorticosteroids (15), and possibly  $\beta_2$ -agonists (16), may exhibit qualitative differences between guinea pig and human airways that concern their effects on plasma exudation.

In the present study involving healthy subjects, we have examined effects of the NO donor nitroprusside on histamine-induced mucosal exudation of bulk plasma ( $\alpha_2$ -macroglobulin) in the ipsilateral nasal airway. Nasal challenge experiments, using changes in nasal patency as response, assisted us in choosing a large tolerable dose of nitroprusside for this interaction study with histamine.

## Material and methods

### *Subjects and study design*

Six healthy subjects, 22–26 years of age (mean age 25), participated in the study. The subjects had no history of general, allergic, or recent nasal disease, and no history of recent drug treatment. The first part of the study, where effects of nitroprusside on nasal patency were examined, was designed as an open study. This part revealed that nasal administration of nitroprusside was associated with a moderate nasal blockage, as well as with a quite detectable pulsating sensation. The subsequent interaction study between nitroprusside and histamine was carried out as a single-blind and placebo-controlled study. However, the lavage fluid levels of  $\alpha_2$ -macroglobulin were assessed blindly as an objective index of mucosal exudation of plasma. The study was approved by the local ethics committee, and informed consent was obtained.

### *Effects of nitroprusside on nasal patency*

A placebo solution containing saline (9.0 mg/ml) and glucose (20 mg/ml), and two doses of sodium nitroprusside (1.2 and 3.6 mg per nasal cavity dissolved in saline (9.0 mg/ml) and glucose (20 mg/ml)), were administered onto the nasal mucosal surface on three separate occasions. The administrations were given as two consecutive actuations ( $2 \times 60 \mu\text{l}$ )

per nasal cavity with a spray device. Nasal peak expiratory flow (PEF) rates were measured before the administrations and thereafter once every third minute for 15 min with using a nasal PEF meter (Mini-Wright, Clement-Clarke, Harlow, UK). At least 3 days elapsed between each visit.

### *Effects of nitroprusside on histamine-induced mucosal exudation of bulk plasma ( $\alpha_2$ -macroglobulin)*

The nasal mucosal surface was lavaged with saline (9.0 mg/ml) by three consecutive lavages with a nasal pool-device (17). The device contained 14 ml saline, and each lavage was kept in the right nasal cavity for 1 min, and then recovered. These lavages were carried out to remove any  $\alpha_2$ -macroglobulin that might be present on the mucosal surface, and the lavage fluids were discarded. Five minutes after these lavages, the mucosa was lavaged with saline for 5 min, to obtain a baseline sample. Immediately after the 5-min lavage, a placebo solution containing saline (9.0 mg/ml) and glucose (20 mg/ml) or nitroprusside (7.2 mg dissolved in saline (9.0 mg/ml) and glucose (20 mg/ml)) was administered as four consecutive actuations ( $4 \times 60 \mu\text{l}$ ) to the right nasal cavity with a nasal-spray device. Three minutes later, the mucosa was challenged with saline (9.0 mg/ml) or histamine (600  $\mu\text{g}$ ) dissolved in saline administered to the right nasal cavity with a nasal-spray device. Four minutes after this challenge, the mucosa was again lavaged with saline for 5 min. The collected lavage fluids were centrifuged (325 g, 10 min,  $4^\circ\text{C}$ ), and samples were obtained from the supernatant and frozen ( $-20^\circ\text{C}$ ) to await analysis of  $\alpha_2$ -macroglobulin. The treatments and challenges followed a scheme of three visits: placebo treatment followed by histamine challenge, nitroprusside treatment followed by histamine challenge, and nitroprusside treatment followed by saline challenge, respectively. At least 1 week elapsed between each visit. The recovery of the lavage fluids was calculated as the initial weight of the nasal pool-device minus its weight after administration divided by the initial weight.

### *Analysis of $\alpha_2$ -macroglobulin*

The lavage fluids were put in coded vials and the levels of  $\alpha_2$ -macroglobulin were measured with a radioimmunoassay sensitive to 7.8 ng/ml. Rabbit antihuman  $\alpha_2$ -macroglobulin (Dakopatts, Copenhagen, Denmark) was used as antiserum and standard human serum (Behringwerke Diagnostica, Marburg, Germany) as standard. Human  $\alpha_2$ -macroglobulin (Cappel-Organon Teknika, Turnhout, Belgium) was iodinated by the lactoperoxidase method. Tracer and standard (or sample) were

mixed with antiserum before adding goat antirabbit antiserum (Astra Draco, Lund, Sweden). The bound fraction was measured with a gamma counter (Pharmacia, Uppsala, Sweden). The intra- and interassay coefficients of variation ranged between 3.8–6.0% and 3.1–7.2%, respectively.

### Statistics

Differences in nasal PEF rates were examined with the Friedman test and the Wilcoxon signed rank test. Differences in lavage fluid levels of  $\alpha_2$ -macroglobulin were examined with the Wilcoxon signed rank test. Data are presented as mean  $\pm$  SEM. *P*-values less than 0.05 were considered significant.

## Results

Nasal nitroprusside administration produced a dose-dependent reduction in nasal PEF rates (Fig. 1). This response was significant at observations 3, 6, and 9 min after nitroprusside administration (Friedman test  $P < 0.05$ , 0.01, and 0.05, respectively, Wilcoxon signed rank test  $P < 0.05$  for comparison between placebo and either nitroprusside dose at these time points). The preadministration baseline nasal PEF rates were regained 15 min after either nitroprusside dose. Nasal administration of nitroprusside was also associated with a pulsating nasal sensation, but no untoward systemic effect could be observed (2.4–7.2 mg total body dose).

Histamine increased the lavage fluid levels of  $\alpha_2$ -macroglobulin ( $P < 0.05$ ) (Table 1). This response was significantly reduced in all subjects when they were given nitroprusside prior to the challenge ( $P < 0.05$ ) (Table 1). Nitroprusside itself did not produce significant luminal entry of  $\alpha_2$ -macroglobulin ( $P = 0.08$ ) (Table 1), although five of the six subjects examined presented somewhat greater lavage fluid

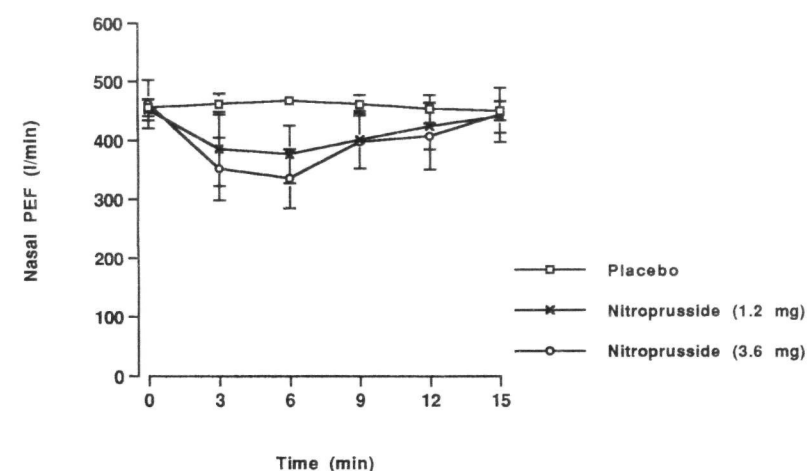


Fig. 1. Effects of nitroprusside (1.2 and 3.6 mg per nasal cavity) on nasal PEF rates. Nitroprusside produced dose-dependent reduction in nasal PEF rates.

Table 1. Levels of  $\alpha_2$ -macroglobulin in nasal lavages obtained before and after treatment/challenge with placebo/histamine, nitroprusside/histamine, and nitroprusside/saline, respectively. Histamine produced mucosal exudation of plasma ( $\alpha_2$ -macroglobulin) ( $P < 0.05$ , comparison between levels before and after placebo treatment and histamine challenge). This response was reduced in all subjects by treatment with nitroprusside ( $P < 0.05$ , comparison between levels after treatment/challenge with placebo/histamine and nitroprusside/histamine). Nitroprusside alone increased lavage fluid levels of  $\alpha_2$ -macroglobulin, but this response failed to reach statistical significance

| Treatment     | Challenge | $\alpha_2$ -macroglobulin ( $\mu\text{g/ml}$ ) |                           |
|---------------|-----------|------------------------------------------------|---------------------------|
|               |           | Before treatment/challenge                     | After treatment/challenge |
| Placebo       | Histamine | 1.33 $\pm$ 0.48                                | 12.2 $\pm$ 2.62           |
| Nitroprusside | Histamine | 0.33 $\pm$ 0.12                                | 6.18 $\pm$ 1.56           |
| Nitroprusside | Saline    | 0.55 $\pm$ 0.36                                | 4.14 $\pm$ 1.44           |

levels of  $\alpha_2$ -macroglobulin after nitroprusside administration.

The recovery of the lavage fluids (nasal pool weight) was  $90 \pm 3$ ,  $94 \pm 2$ , and  $94 \pm 1\%$ , respectively, at the lavages that were carried out before the treatments/challenges at the three visits. The recovery was  $95 \pm 3$ ,  $94 \pm 1$ , and  $94 \pm 2\%$  after treatment/challenge with placebo/histamine, nitroprusside/histamine, and nitroprusside/saline, respectively.

## Discussion

This study has shown that topical administration of nitroprusside has the ability to reduce mucosal exudation of plasma induced by histamine in the human nasal airway. This result, together with animal data (11), suggest that topical NO-donor drugs may exert a degree of antiexudative effect in both guinea pig and human airways.

We have previously shown by analyses of nasal lavage fluid levels of albumin, fibrinogen, and  $\alpha_2$ -macroglobulin that histamine-induced luminal entry of  $\alpha_2$ -macroglobulin corresponds to mucosal exudation of lightly sieved "bulk" plasma (18). Importantly, extravasated plasma may not be retained in the airway tissue because its luminal entry extends to threshold inflammatory responses. The mechanisms and the ease by which the extravasated plasma enters the airway lumen (1, 5) suggest that the antiexudative effects of nitroprusside shown in this study may not be an effect on the epithelial barrier function, but rather on the permeability of the subepithelial microcirculation.

Kuo et al. (19) reported that intravenous administration of L-NAME may inhibit neurogenic plasma exudation responses in guinea pig airways and suggested that endogenous NO is involved in generating exudative inflammation in the airways. In contrast, we have observed that topical administration of L-NAME produces plasma exudation in guinea

pig airways (6). This difference between topical and intravenous L-NAME has not been explained. It is possible that the use of topical L-NAME generates information about the activity of superficial, presumably epithelial, NO systems, whereas the systemic L-NAME may produce an airway effect that involves primary effects of L-NAME outside the lung airways. It is further possible that the findings by Kuo et al. (19) pertain specifically to neurogenic plasma exudation. Since this latter response appears to be confined to rats and guinea pigs and is not present in human airways, it would not be relevant for the present study (13, 14). Furthermore, topical administration of the NO donor nitroprusside was shown to reduce an inflammatory stimulus-induced plasma exudation response in guinea pigs (11). The present data agree with the possibility that NO donor drugs such as nitroprusside have a capacity to reduce acute plasma exudation responses in human airways. However, from the present single-dose study, the magnitude of the inhibitory effect of nitroprussides on inflammatory stimulus-induced mucosal exudation of plasma cannot be fully assessed. Moreover, it is possible that the tendency of nitroprusside itself to produce plasma exudation (Table 1) may have partly concealed its inhibitory capacity (Table 1).

Certain inflammatory mediators, such as histamine (17) and, particularly, leukotriene D<sub>4</sub> (20), have the ability to produce nasal blockage. This response largely reflects vasodilation and filling of venous sinuses. These agents also induce plasma exudation responses, but edema of the airway tissue may not be pronounced because of luminal entry of the extravasated plasma (21). In this study, the vasodilator drug nitroprusside produced a dose-dependent nasal blockage that lasted for about 10 min. This finding suggests that the vasodilator action of nitroprusside involves filling of nasal mucosal veins. Vasodilation *per se* enhances plasma exudation responses in tissues with poor baseline blood supply, such as the skin (22). In this study, nitroprusside alone tended to increase the lavage fluid levels of  $\alpha_2$ -macroglobulin, although this effect did not reach statistical significance. Although this does not seem likely, it cannot be excluded that vasodilation may have contributed to the tendency of nitroprusside alone to increase the baseline plasma exudation.

In conclusion, this study has demonstrated that topical nitroprusside may have antiexudative effects in human airways. This finding is of interest in view of previous failures to translate guinea pig airway data on vascular permeability active principles into human airway relevance. Further studies are required to determine whether NO donor drugs may be a useful antipermeability principle in human airways.

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