

## Effects of two weeks of topical budesonide treatment on microvascular exudative responsiveness in healthy human nasal airways

L. Greiff\*, M. Andersson\*, C. Svensson\*, A. Åkerlund\*, U. Alkner\*\*, C.G.A. Persson+

*Effects of two weeks of topical budesonide treatment on microvascular exudative responsiveness in healthy human nasal airways. L. Greiff, M. Andersson, C. Svensson, A. Åkerlund, U. Alkner, C.G.A. Persson. ©ERS Journals Ltd 1997.*

**ABSTRACT:** Extravasation and luminal entry of plasma (mucosal exudation) is not only a key feature of airway inflammation in rhinitis and asthma but also a major first-line respiratory defence mechanism. Topical steroids are effective anti-exudative agents in disease but, so far, little is known about the direct effects of these drugs on the responsiveness of the microcirculation in human airways.

In this study, the effects of prolonged budesonide treatment on histamine-induced mucosal exudation of plasma was examined in 42 healthy subjects. Placebo and budesonide (100 µg per nasal cavity *b.i.d.*) were given for 2 weeks in a double-blind and placebo-controlled parallel-group protocol. Using a nasal pool technique, nasal challenges with isotonic saline and histamine (40 and 400 µg·mL<sup>-1</sup>) were carried out before and late in the treatment periods. The lavage fluid levels of α<sub>2</sub>-macroglobulin were measured as an index of mucosal exudation of bulk plasma.

Histamine produced concentration-dependent mucosal exudation of plasma before as well as after treatment with either placebo or budesonide. The topical steroid treatment only marginally (1.8 fold) decreased the response to the low concentration histamine (40 µg·mL<sup>-1</sup>) and, although it was significantly (2.8 fold) reduced, histamine 400 µg·mL<sup>-1</sup> still produced significant mucosal exudation of plasma in the budesonide group.

If the present observations are extrapolated to inflammatory conditions, the antiexudative effects of topical steroids in rhinitis (and asthma) may reflect only a small degree of microvascular antipermeability effects. We suggest that topical steroid treatment may not impede mucosal exudation responses when called for in acute human airway defence reactions.

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Depts of \*Otorhinolaryngology, Head and Neck Surgery and +Clinical Pharmacology, Lund University Hospital, Lund, Sweden.  
\*\*Dept of Bioanalysis, Astra-Draco, Lund, Sweden.

Correspondence: L. Greiff  
Dept of Otorhinolaryngology, Head and Neck Surgery  
Lund University Hospital  
S-221 85 Lund  
Sweden

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Microvascular-epithelial exudation of plasma (mucosal exudation of plasma) may reflect the intensity and time course of significant inflammatory processes in the airways [1]. Supporting this notion, treatment with topical airway steroids reduces exudation in diseases, such as rhinitis and asthma, where plasma exudation is increased [2, 3]. In the airways of healthy guinea-pigs and rats [4–6], as well as in the hamster cheek pouch preparation [7], glucocorticosteroids may completely abolish the acute effects of inflammatory histamine-type mediators. The microvascular antipermeability effect of topical glucocorticoids in these animal studies is very pronounced within an hour, and lasts for several hours even after a single brief tissue exposure to the glucocorticoid. If this effect of abolishing the microvascular end-organ's ability to respond also occurs in human airways, treatment with steroids would be potentially harmful because extravasation and exudation of bulk plasma, a major defence process [8], would be inhibited.

However, in acute experiments involving healthy humans, a single clinical dose of the glucocorticoid budesonide did not affect histamine challenge-induced mucosal exudation of plasma [9]. Further studies in human airways now appear to be warranted in order to determine whether prolonged topical treatment with glucocorticoid drugs, rather than a single dose [10], may produce airway microvascular antipermeability effects. This aspect is of importance because extravasation and luminal entry of plasma and its derived multipotential effector solutes is an airway defence mechanism in health and disease [8], that should not be inhibited indiscriminately.

Since the hypothesis-generating studies in this area of steroid pharmacology were carried out in the airways of healthy animals, it appears proper to extend the study of this phenomenon to healthy human subjects. Basic information in healthy airways is also warranted in view of potential confounding factors in disease relating to the contributory actions of steroids on the cellular inflammatory processes (that evoke exudation) and on the exudative hyperresponsiveness that has been demonstrated in allergy and infection [11].

In the present study, involving healthy subjects, we

have examined the effects of 2 weeks of treatment with a clinically significant daily dose of budesonide (200 µg per nasal cavity) [10] on histamine-induced mucosal exudation of bulk plasma. To elucidate whether any anti-permeability actions of the steroid would be dependent on the magnitude of the exudative stimulus, we employed challenges with two effective but 10 fold different concentrations of histamine. The present work, thus, examines the possibility that maintenance treatment with topical nasal steroids may affect microvascular exudative responsiveness in healthy airways.

## Methods

### Study design

The present study was of a double-blind, placebo-controlled, and parallel-group design. Histamine-challenges were performed before and after 2 weeks of topical treatment with either the glucocorticosteroid, budesonide, or placebo. The levels of  $\alpha_2$ -macroglobulin (725 kDa) were measured in lavage fluids obtained after challenge with isotonic saline and histamine, respectively, as an index of mucosal exudation of plasma. In order to avoid the influence of interindividual variations in the nasal mucosal exudative responsiveness to histamine, the primary statistical comparison was chosen to be paired within each treatment group.

### Subjects

Forty two healthy subjects, 20–27 yrs of age (mean age 24 yrs), participated in the study. The subjects were nonsmokers and had no history of general, allergic (verified by a negative skin-prick test), or recent nasal disease, and no history of recent drug treatment. The study was approved by the local ethics committee, and informed consent was obtained.

### Budesonide treatment

Budesonide aqueous suspension (100 µg per nasal cavity) or placebo was administered twice daily using a nasal pump spray device. The subjects were provided with an allocation number, and the order of treatment proceeded according to a randomization scheme of the allocation numbers prepared in blocks. Blindness was maintained by the identical appearance of active and placebo drug delivery devices. Instructions were given to the subjects regarding the handling of the spray device and the first administration was supervised.

### Challenges and lavages

A nasal pool device was used for concomitant challenge and lavage of the nasal mucosa [12]. The nasal pool device is a compressible plastic container equipped with a nasal adapter. The adapter is inserted into one

of the nostrils and the container is compressed by the sitting subject, 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 area of the mucosal surface for a selected period of time. When the pressure on the device is released, the fluid returns into the container. In the present study, the volume of the fluid was 14 mL. Using the nasal pool technique, control isotonic saline and histamine challenges were performed prior to and 2 weeks into the budesonide or placebo treatment.

In previous experiments, we have demonstrated that 40–2,000 µg·mL<sup>-1</sup> is the effective concentration range in which histamine produces significant and concentration-dependent plasma exudation responses, when administered to the human nasal airway using the nasal pool technique [12]. In the present study, isotonic saline and histamine (40 and 400 µg·mL<sup>-1</sup>) in isotonic saline were introduced into the right nasal cavity as three consecutive administrations using nasal pool devices. The duration of each challenge and lavage was 10 min. Prior to each 10 min instillation, the mucosal surface was irrigated by two consecutive 30 s saline lavages to remove any  $\alpha_2$ -macroglobulin that might have accumulated on the mucosal surface. Furthermore, to prevent histamine from being retained in the nasal airway, the mucosal surface was irrigated by a 30 s saline lavage, using the nasal pool technique, immediately after each 10 min challenge. These brief lavages were not collected. The recovered lavage fluids were centrifuged (105×g for 10 min at 4°C) and aliquots were prepared from the supernatants and frozen (-20°C) for later analysis of  $\alpha_2$ -macroglobulin.

### Analysis of $\alpha_2$ -macroglobulin

The lavage fluids were placed in coded vials and the levels of  $\alpha_2$ -macroglobulin were measured using a radioimmunoassay sensitive to 7.8 ng·mL<sup>-1</sup>. 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 using 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 using a gamma counter (Pharmacia, Uppsala, Sweden). The intra- and interassay coefficients of variation were between 3.8–6.0 and 3.1–7.2%, respectively.

### Statistics

Differences in  $\alpha_2$ -macroglobulin levels prior to and after treatment were analysed by Wilcoxon signed rank test. Differences in  $\alpha_2$ -macroglobulin within each challenge series were analysed first by Friedman test and, if a statistical significance emerged, by Wilcoxon signed rank test. A p-value less than 0.05 was considered significant. Data are presented as mean±SEM.

Table 1. – Levels of  $\alpha_2$ -macroglobulin in lavages obtained after challenge with saline and histamine (40 and 400  $\mu\text{g}\cdot\text{mL}^{-1}$ ) before and after treatment with placebo (n=21) and budesonide (n=21)

Challenge	$\alpha_2$ -macroglobulin $\mu\text{g}\cdot\text{mL}^{-1}$			
	Placebo treatment		Budesonide treatment	
	Before	After	Before	After
Saline	0.4±0.2	0.8±0.3**	0.5±0.3	0.2±0.1
Histamine 40 $\mu\text{g}\cdot\text{mL}^{-1}$	4.2±1.6	8.8±6.1	1.9±0.5	1.1±0.3
Histamine 400 $\mu\text{g}\cdot\text{mL}^{-1}$	20.8±6.7	17.2±3.2	9.1±2.4	3.2±0.9**

Values are presented as mean±SEM. \*\*: p<0.01, compared to level before treatment.

Results

Histamine produced concentration-dependent mucosal exudation of plasma ( $\alpha_2$ -macroglobulin) before the treatments (table 1). This effect was significant for histamine (40 and 400  $\mu\text{g}\cdot\text{mL}^{-1}$ ) in the groups that were to receive treatment with placebo as well as budesonide (Friedman tests, p<0.0001; Wilcoxon signed rank tests, p<0.0001) (figs 1 and 2).

The responsiveness to histamine (400  $\mu\text{g}\cdot\text{mL}^{-1}$ ) was significantly reduced by treatment with budesonide (p<0.01) (fig. 2c), whereas it was unaffected by placebo treatment (p=0.6) (fig. 1c). However, the exudative responsiveness was not completely abolished, as histamine (400  $\mu\text{g}\cdot\text{mL}^{-1}$ ) still produced significant mucosal

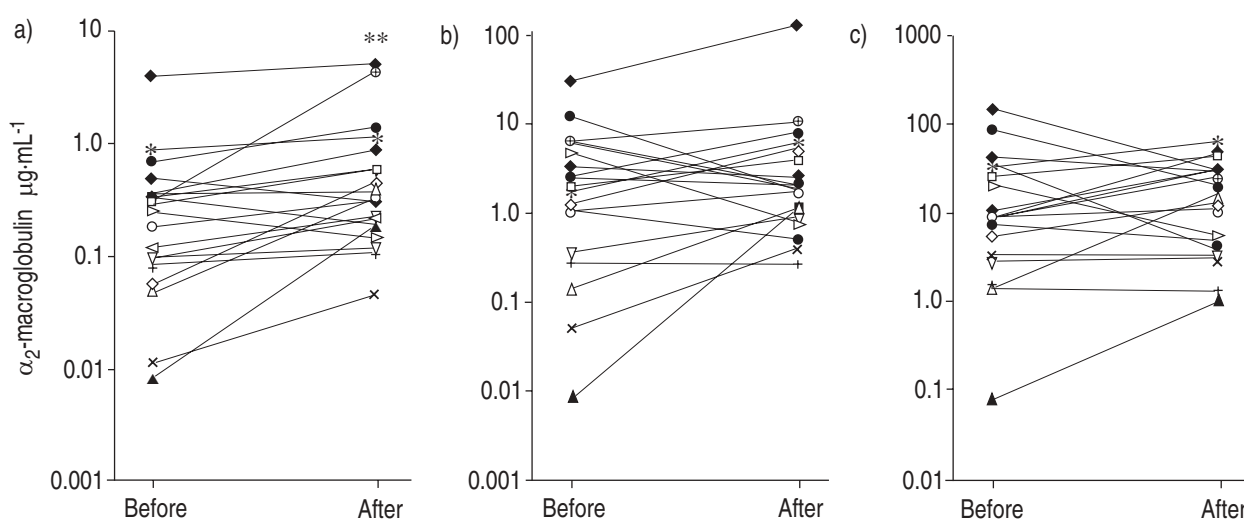


Fig. 1. – Effects of topical placebo on nasal lavage fluid levels of  $\alpha_2$ -macroglobulin after challenge with: a) control saline; b) histamine 40  $\mu\text{g}\cdot\text{mL}^{-1}$ ; and c) histamine 400  $\mu\text{g}\cdot\text{mL}^{-1}$ , performed before and after treatment. Paired data are connected. Placebo did not reduce the baseline levels of  $\alpha_2$ -macroglobulin or the histamine-induced mucosal exudation of plasma ( $\alpha_2$ -macroglobulin) \*\*: p<0.01, compared to the level before treatment.

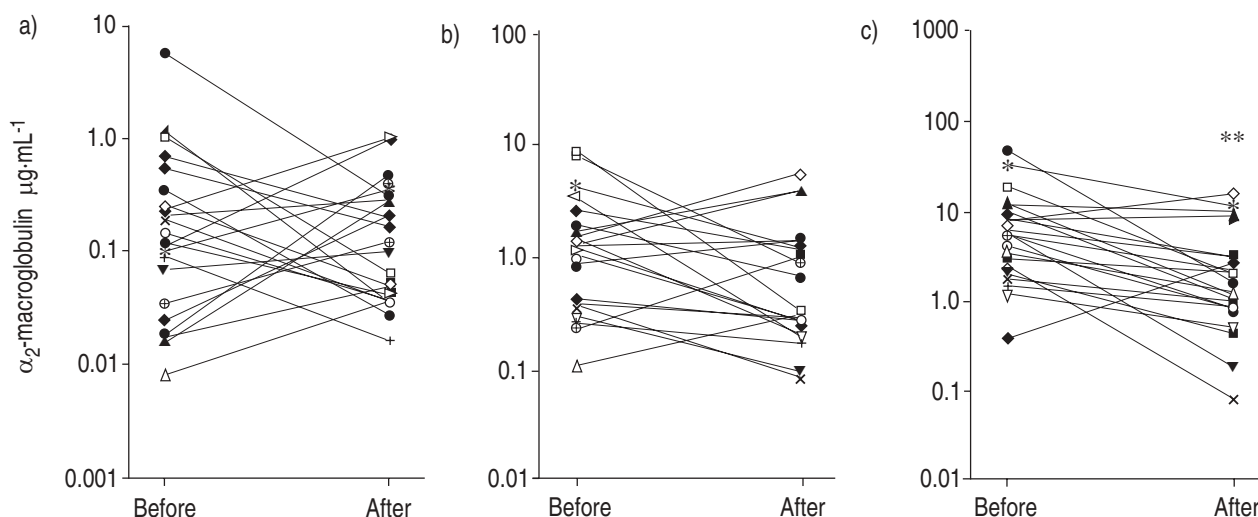


Fig. 2. – Effects of topical budesonide (100  $\mu\text{g}$  per nasal cavity administered *b.i.d.*) on nasal lavage fluid levels of  $\alpha_2$ -macroglobulin after challenge with: a) control saline; b) histamine 40  $\mu\text{g}\cdot\text{mL}^{-1}$ ; and c) histamine 400  $\mu\text{g}\cdot\text{mL}^{-1}$ , performed before and after treatment. Paired data are connected. Budesonide reduced the histamine 400  $\mu\text{g}\cdot\text{mL}^{-1}$  induced mucosal exudation of plasma ( $\alpha_2$ -macroglobulin), but did not reduce the baseline levels of  $\alpha_2$ -macroglobulin or the histamine 40  $\mu\text{g}\cdot\text{mL}^{-1}$  induced mucosal exudation of  $\alpha_2$ -macroglobulin. \*\*: p<0.01, compared to the level before treatment.

exudation of plasma (Friedman test,  $p < 0.0001$ ; Wilcoxon signed rank test,  $p < 0.0001$ ) after topical budesonide treatment. The responsiveness to histamine ( $40 \mu\text{g}\cdot\text{mL}^{-1}$ ) was not affected by the placebo (Wilcoxon signed rank test,  $p = 0.1$ ) or the budesonide treatment (Wilcoxon signed rank test,  $p = 4$ ) (figs. 1b and 2b). The baseline appearance of  $\alpha_2$ -macroglobulin on the nasal mucosa was slightly increased in the group receiving placebo (Wilcoxon signed rank test,  $p < 0.01$ ). This change did not occur in subjects receiving budesonide (figs. 1a and 2a).

### Discussion

The present study, involving 42 healthy subjects, has demonstrated that 2 weeks of treatment with topical budesonide may only marginally attenuate the plasma exudation response to a challenge with histamine in human nasal airways. This observation is important in view of the role of plasma exudation responses in respiratory defence. It may also shed some light on mechanisms involved when steroids exhibit strong inhibition of airway mucosal exudation in bulk plasma.

The physiological, noninjurious mechanisms involved in extravasation and luminal entry of bulk plasma in animal and human airways have recently prompted the proposal that mucosal exudation should be considered a major first-line defence of the airway mucosa [8]. After extravasation from the subepithelial microcirculation, there is a brief and 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 [13]. Gently, through a sensitive hydraulic mechanism (a basolateral pressure of less than  $5 \text{ cmH}_2\text{O}$  seems sufficient) the exudate makes ubiquitous paracellular pathways into the airway lumen [14]. Although this process involves movement of bulk plasma, including large plasma proteins, such as  $\alpha_2$ -macroglobulin (determined in the present study), the luminal entry does not damage the epithelium [13]. Moreover, the mucosal exudation process is not associated with any increased absorption, *i.e.* the permeability of the epithelium to hydrophilic molecules that enter the mucosa from the luminal compartment is unaffected [15, 16]. The self-sustained epithelial transmission of plasma exudates seems to exclude the epithelial barrier-function as an important pharmacological target for steroids and other antiexudative agents. Indeed, a selective epithelial tightening effect would not be desirable in exudative conditions, because it would promote oedema formation by inhibiting a major clearance route for the tissue plasma exudate [17]. Furthermore, the present results question the importance of the reputed direct microvascular antipermeability effect of steroids in human airways.

The mucosal exudation response to histamine involves direct effects on the superficial airway microvessels. Moreover, histamine appears to be without any action on the epithelial value-like barrier function that allows luminal entry of macromolecules at slightly increased basolateral hydrostatic pressures [18]. Histamine is believed to increase venular permeability to macromolecules by inducing distinct endothelial cell "separation" effects [19, 20]. The venular endothelium is also a pos-

sible target for the present effect of budesonide, attenuating the exudation response to  $400 \mu\text{g}\cdot\text{mL}^{-1}$  histamine. Other targets, including effects on blood flow appear more remote: hypothetically, a decreased blood flow could reduce the exudation, but topical budesonide has little effect on nasal blood flow [21]. Furthermore, baseline blood flow of the mucosal microcirculation is so rich that even significant decongestant doses of a topical  $\alpha$ -receptor agonist (oxymetazoline) are without effect on the exudative responsiveness to histamine-type mediators in the human nose [22].

The present antiexudative effect is not marked, such as that seen with budesonide in allergic airways disease. Moreover, only the effects of the larger dose of histamine were significantly attenuated in this study. This observation remains unexplained and need, to be confirmed. It may reflect a general suppressive action by steroid treatment, as also suggested by an overall non-significant tendency to increased response after placebo treatment and an opposite trend in the steroid group. The limited efficacy is particularly evident from the present interaction between prolonged budesonide treatment and histamine given at a dose belonging to the lower concentration response line to this agent. Hence, prolonged treatment with a relatively large clinical dose of a potent topical steroid [10] may not deprive the mucosal microcirculation of its ability to launch plasma exudation responses in airway defence. The present observation concurs with a lack of effect of topical airway steroids to reduce exudation during several acute airway events, including viral infection [23], epithelial damage-restitution processes [24], and exposure to toxic chemicals [25]. The present data indicating a potentially well-maintained mucosal defence mechanism further agree with clinical observations of unchanged or reduced rates of airway infections in patients with asthma and rhinitis receiving chronic therapy with airway steroids [10]. Studies on the antiexudative effects of typical steroids in patients with inflammatory airway disease now seem warranted.

In conclusion, this study, involving healthy volunteers, has demonstrated that 2 weeks of treatment with a potent airway steroid may reduce but may not abolish airway exudation responses to challenge with acute exudative agents.

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