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Glucocorticoids may not inhibit plasma exudation by direct vascular antipermeability effects in human airways

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

Glucocorticoids may not inhibit plasma exudation by direct vascular antipermeability effects in human airways. L. Greiff, M. Andersson, C. Svensson, U. Alkner, C.G.A. Persson. ©ERS Journals Ltd 1994.

ABSTRACT: In animal airways, single topical treatment with glucocorticoids produces a prompt vascular anti-permeability effect that may last for several hours. This has been considered a potentially important anti-inflammatory action in human airways. The present study, involving nine healthy subjects, examines whether nasal budesonide application affects histamine-induced mucosal exudation of plasma and plasma-derived mediators in human airways.

A selected low concentration (40 μg·ml^{-1}) of the vascular permeability-inducing agent histamine was kept in one of the nasal cavities for 10 min, and this challenge was repeated at 50 min intervals over 4 h. Ten minutes after the first histamine-challenge, a clinical dose of budesonide (100 μg) was sprayed into the same nasal cavity. Nasal lavage fluid levels of albumin and fibrinogen were measured as indices of mucosal exudation of bulk plasma, and bradykinins were analysed to indicate generation of plasma-derived mediators. The baseline levels of albumin and fibrinogen were evaluated in 24 healthy control subjects by means of a 10 min saline lavage.

Histamine produced significant mucosal exudation of albumin and fibrinogen, compared to control subjects. Topical budesonide treatment did not affect the histamine-induced mucosal exudation of albumin or fibrinogen, nor did budesonide affect the mucosal output of bradykinins.

The present human airway data do not support the view, based on animal findings, that airway glucocorticoids reduce mucosal exudation of plasma by direct vascular effects. We suggest that anti-exudative effects of topical glucocorticoids in airway diseases indirectly reflect the inhibition of cellular inflammatory processes by these drugs, rather than any direct effects on the airway microcirculation.

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Depts of *Otorhinolaryngology and +Clinical Pharmacology, Lund University Hospital, Lund, Sweden. **Dept of Bioanalysis, Astra Draco, Lund, Sweden.

Correspondence: L. Greiff
Dept of Otorhinolaryngology
Lund University hospital
S-221 85 Lund
Sweden

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In rhinitis and asthma, luminal entry of plasma (mucosal exudation of plasma) may reflect the intensity and the time-course of the airway inflammatory processes [1]. Airway challenge with allergen, occupational agents, and other factors that activate disease-like cellular processes, also induce the plasma exudation response [1]. Prompt and transient mucosal exudation of plasma is evoked by histamine-type inflammatory mediators, that probably act directly on endothelial cells of the microvascular wall [2]. Antiexudative drugs that prevent the action of these directly-acting mediators are identified as vascular antipermeability drugs [2].

Treatment of inflammatory airways disease with topical steroids results in inhibition of mucosal exudation of plasma [3, 4]. It is not known whether this effect reflects the ability of steroids to inhibit important cellular inflammatory processes, or whether steroids may act directly on the microvascular end-organ in human airways. In the airways of guinea-pigs [5], and rats [6], and in the hamster cheek pouch preparation [7], steroid treatment attenuated the exudative effect of directly-acting inflammatory mediators. The microvascular antipermeability effects of topical steroids in the animal experiments is pronounced within an hour and lasts for several hours, even after brief exposure of the tissue to the steroid.

In the present study, involving healthy subjects, we have examined effects of a single nasal application of budesonide, a widely-used anti-rhinitis and anti-asthma glucocorticoid, on histamine-induced mucosal exudation of plasma (albumin and fibrinogen) and plasma-derived peptides (bradykinins). To provide optimum sensitivity to any antipermeability action of the steroid we have used an exudative challenge dose of histamine in the lower part of the concentration-response line to histamine, in the human nasal airway [8].

Methods

Subjects

Nine healthy subjects, 20–27 yrs of age (mean age 24 yrs), received histamine-challenges and budesonide treatment.

The subjects had no history of general, allergic 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.

Nasal pool-technique

A nasal pool-device was used for concomitant histamine-challenge and lavage of the nasal mucosa [8]. It was also used for saline lavage in the evaluation of baseline levels of albumin and fibrinogen in nasal mucosal surface liquids. 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 subjects leaning forward in a 60° flexed neck position. The nasal pool-fluid is then instilled into one nasal cavity and maintained in contact with a large and reproducible 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. In the present study, the volume of the nasal pool-fluid was 14 ml.

Histamine-induced mucosal exudation of plasma

In previous experiments, we have demonstrated that 40–2,000 $\mu m \cdot ml^{-1}$ (2.2×10^{-4}–110×10^{-4} M) is the effective concentration range, in which histamine produces significant and concentration-dependent plasma exudation responses in the human nasal airway [8]. In the present study, histamine (40 $\mu g \cdot ml^{-1}$) in saline (9.0 $mg \cdot ml^{-1}$) was introduced into the right nasal cavity on five consecutive occasions, using a nasal pool-device. The duration of each challenge was 10 min, and 50 min elapsed between each administration. The lavage fluid concentrations of albumin, fibrinogen and bradykinins were measured as indices of mucosal exudation of plasma. In separate experiments, 24 healthy subjects, 20–24 yrs of age (mean age 22 yrs), were exposed to saline using the same technique as with histamine. Thus, we obtained control baseline values of albumin and fibrinogen in the lavage fluids.

Experimental design

A clinical dose of budesonide (100 µg) was administered to the right nasal cavity as a single actuation 10 min after the first histamine-challenge, using a nasal spray-device. Budesonide is a highly fat soluble molecule, with a rapid uptake and high affinity to airway tissue, from which it is not readily washed away by lavage fluids or exudation processes [9]. Hence, the repeated lavages and induced exudation in this study would not impede the possibility for budesonide to exert any subepithelial microvascular effect. The budesonide treatment followed a randomized, double-blind, cross-over and placebo-controlled design. At least one week elapsed between the first and second treatment.

Analyses of albumin, fibrinogen and bradykinins

The recovered lavage fluids were centrifuged (325×g, 10 min, 4°C) and samples of the supernatant obtained and frozen (-20°C) to await analysis. Ethylenediamine tetra-acetic acid (EDTA) (0.2 M, 200 µl) was added to separate 800 µl lavage fluid samples awaiting analysis of bradykinins. Albumin was measured by a radio-immunoassay sensitive to 6.25 $ng \cdot ml^{-1}$. Human albumin (Calbiochem, San Diego, CA, USA) was used as reference, and anti-albumin (Dakopatts, Copenhagen, Denmark) and anti-immunoglobulin G (IgG) (Astra-Draco, Lund, Sweden) for detection. The intra- and interassay coefficients of variation were 5 and 10%, respectively. Fibrinogen was measured by a radio-immunoassay sensitive to 2 $ng \cdot ml^{-1}$. Human fibrinogen (Sigma, St. Louis, MI, USA) was used as reference, and rabbit anti-human fibrinogen (UCB, Brussels, Belgium) and goat anti-rabbit IgG (Astra-Draco, Lund, Sweden) for detection. The intra- and interassay coefficients of variation were 7 and 12%, respectively. Bradykinins (bradykinin and lysyl-bradykinin) were measured by a radio-immunoassay sensitive to 20 $pg \cdot ml^{-1}$. Bradykinin (Bachem Feinchemikalien, Bubendorf, Switzerland) was used as reference, and anti-bradykinin (Peninsula Lab, Belmont, CA, USA) and anti-IgG (Glostrup Hospital, Denmark) for detection. The intra- and interassay coefficients of variation were 7 and 12%, respectively. The cross-reactivity with lysyl-bradykinin was 40%.

Statistics

Differences in albumin and fibrinogen levels between control and histamine-challenged subjects were analysed by Mann-Whitney U-test. Difference in albumin, fibrinogen and bradykinin levels between histamine-challenged subjects prior to placebo/budesonide treatment were analysed by Wilcoxen signed rank test. Differences in albumin, fibrinogen and bradykinin levels between histamine-challenges subjects with and without budesonide treatment were analysed by Wilcoxen signed rank test of cumulative levels (from the second to the fifth observation). Differences within each treatment group were analysed by Friedman test and Wilcoxon signed rank test. A p-value less than 0.05 was considered significant. Data are presented as mean±SEM.

Results

Histamine (40 $\mu g \cdot ml^{-1}$) produced significant mucosal exudation of albumin and fibrinogen compared to control subjects: the levels of albumin were 77.7±15.3 $\mu g \cdot ml^{-1}$ in histamine-challenged subjects (prior to budesonide treatment), and 16.8±3.2 $\mu g \cdot ml^{-1}$ in control subjects ($p<0.001$). The levels of fibrinogen were 3,087±762.5 $ng \cdot ml^{-1}$ in histamine-challenged subjects and 117±85.0 $ng \cdot ml^{-1}$ in control subjects ($p<0.001$).

The lavage fluid concentrations of albumin and fibrinogen, as well as bradykinins were not reduced by budesonide ($p>0.05$) (fig. 1 and table 1). The histamine-induced

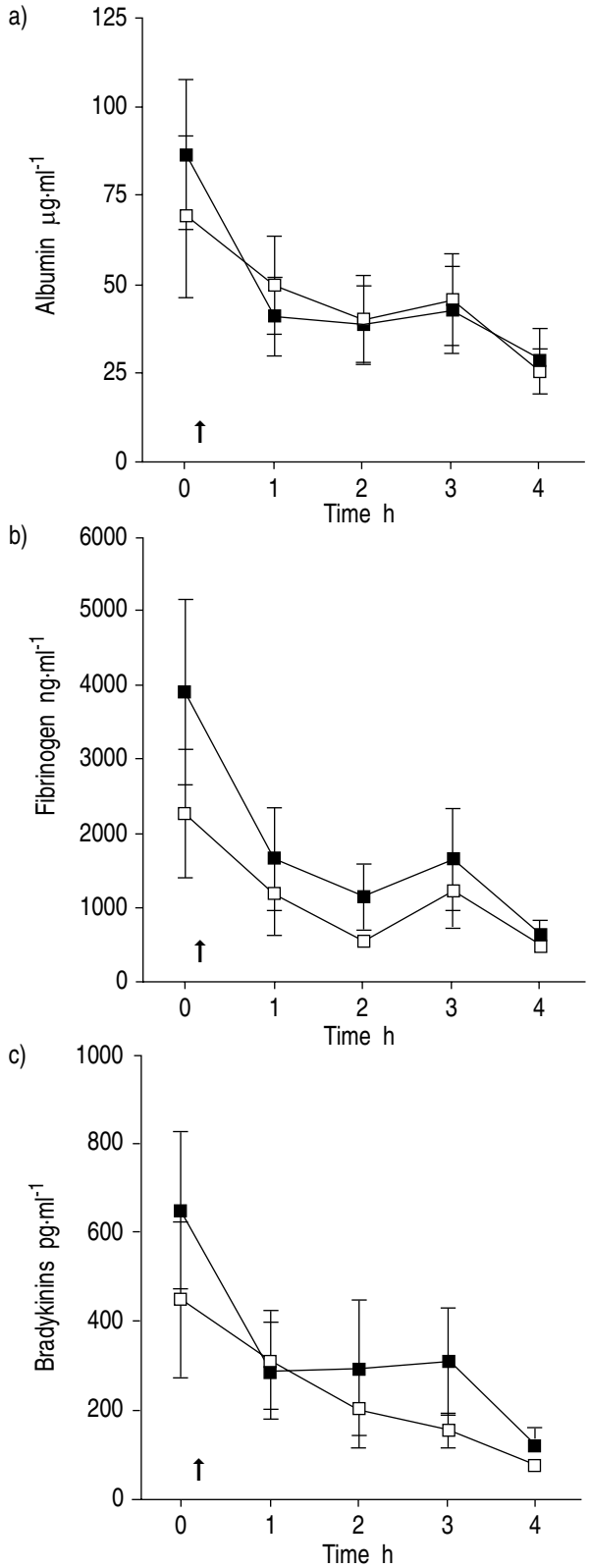

Fig. 1. – Effects of single topical budesonide-treatment (100 µg) and placebo-treatment, respectively, (indicated by arrows) on mucosal exudation of: a) albumin; and b) fibrinogen; and c) mucosal output of bradykinins, induced by a threshold exudative histamine-challenge (40 µg·ml^{-1}) repeated once every hour for 4 h. The lavage fluid concentrations of albumin, fibrinogen and bradykinins were not significantly different between placebo and budesonide treated subjects. —□—: placebo; —■— : budesonide.

Table 1. – Cumulative levels of albumin, fibrinogen and bradykinins, respectively, calculated by addition of lavage fluid levels for the second to the fifth observation, *i.e.* the observations made after placebo and budesonide treatment: comparisons between placebo and budesonide treatment

	Placebo	Budesonide	p-values
Albumin µg·ml^{-1}	158±39.8	149±38.1	0.31
Fibrinogen ng·ml^{-1}	3356±1125	4998±1779	0.14
Bradykinins pg·ml^{-1}	733±205	993±395	0.68

lavage fluid levels of albumin, fibrinogen and bradykinins were not significantly different between the subjects prior to treatment ($p>0.05$).

The frequent histamine-challenges produced a repeatable but successively decreased exudative response (fig. 1). After placebo treatment, the decrease was significant with albumin (Friedman; $p<0.05$), fibrinogen (Friedman; $p<0.01$), and bradykinins (Friedman; $p<0.05$). Also, after treatment with budesonide the histamine-induced exudation was decreased by repeated challenges (Friedman; albumin $p<0.01$, fibrinogen $p<0.01$, bradykinins $p<0.001$). The decrease was significant between the first and the fifth histamine-challenge after placebo as well as budesonide treatment in all groups (Wilcoxon; $p<0.05$–0.01). There was no tendency that the budesonide treatment had increased the decline of the exudation responses to histamine.

Discussion

The present study demonstrates that a single topical budesonide administration had no effect on the mucosal exudation of plasma and the mucosal output of bradykinins, induced by the vascular permeability increasing agent, histamine. The present findings do not support the current view, based on findings in animals, that topical steroids have direct actions on the airway microvascular permeability. Nonvascular anti-inflammatory mechanisms may explain the antiexudative efficacy of glucocorticoids in rhinitis and asthma.

Inflammatory mediators, including histamine, produce active separation of the endothelial cells of post-capillary venules of the subepithelial airway microcirculation. Through gaps in the venular wall, nonsieved bulk plasma is extravasated along a hydrostatic pressure gradient. The extravasated plasma moves up between epithelial cells and produces paracellular pathways for its clearance into the airway lumen [1, 10, 11]. The plasma extravasation process in the airways can, thus, be monitored by analysing the concentrations of plasma proteins in airway mucosal surface liquids [1]. The present study confirmed the exudative effects of histamine, and demonstrated that repeated histamine-challenges produced successively decreased lavage fluid levels of plasma proteins, indicating a degree of tachyphylaxis of the response to 40 µg·ml^{-1} of histamine. This differs from previous observations, demonstrating well-maintained mucosal exudation of plasma in the human nasal airway after topical

administration of a high dose of histamine (1.0 mg) given at 30 min intervals [12]. It is conceivable that a small degree of tachyphylaxis may not be detected when large doses are employed, whereas it may become evident when a "threshold" effective dose is used.

The mechanism behind the present observation on tachyphylaxis to histamine may be similar to that observed in the hamster cheek pouch preparation [2]. However, it is also in the latter type of preparations that the direct antipermeability effect of budesonide and dexomethasone has been most clearly demonstrated. SVENSJÖ and ROEMPKE [7], thus, exposed the oral microcirculation, freed from epithelium, to budesonide for brief periods of time (5–30 min). This treatment inhibited subsequent extravasation responses produced by histamine-challenges which were repeated every 30 min for 3 h. Hence, the present study design copied that previously employed in the animal experiments.

Clinical trials with intranasal budesonide, given at a total daily dose of 400 µg, have demonstrated that it is clinically effective in seasonal and perennial allergic rhinitis [13]. Budesonide also reduced the numbers of eosinophils in nasal brush specimens [14], and the lavage fluid concentrations of fibrinogen and bradykinins [3], in patients with seasonal allergic rhinitis. The latter observation indicates that topical budesonide reduces allergen-induced mucosal exudation of plasma. The daily dose of budesonide can be given once a day, or it can be given twice a day with 100 µg per nasal cavity [13].

Studies involving animal tracheobronchial airways have demonstrated that a single airway administration of a glucocorticoid may reduce, or even abolish, mucosal exudation of plasma induced by the histamine type mediators [5, 6]. Hence, glucocorticoids may exert antiexudative effects by a direct action on the endothelial cells of postcapillary venules in animals. In contrast, the present data did not show the slightest tendency of reduction of the histamine-induced effects by budesonide. Hence, topical glucocorticoids may not produce direct antiexudative actions on the subepithelium microcirculation of the human nasal airway. The study also showed that an acute treatment with a topical steroid may not prevent the formation of bradykinins from kininogens, which are contained in the bulk plasma exudate.

It is difficult to compare topical steroid levels between species, since the effective dose may differ greatly. For the present experiments, we selected 100 µg of budesonide. This is a clinically relevant dose and also a relatively large glucocorticoid dose to be applied on only half the nasal mucosal surface area [13]. The antipermeability effects in animal cheek pouch and tracheal microcirculation was observed after exposure to a low concentration (10^{-7} M) of budesonide [6, 7], suggesting that in animals the microvascular endothelial cells are sensitive to glucocorticoid action. We also employed a relatively low concentration (approximately the concentration producing 20% maximal effect (EC_{20})) of histamine in this study, so that a large exudative response, that could blunt a weak antiexudative property of the steroid treatment, was avoided. The challenge concentrations of histamine and bradykinin employed in the animal experiments were approximately the concentration producing half the maximal effect (EC_{50}) [6, 7]. The choice of drug and challenge doses in this study strengthen the conclusion that animal and human airway microvessels may differ qualitatively with respect to glucocorticoid-induced effect on vascular permeability.

It cannot be excluded that topical budesonide produces a degree of vasoconstriction in the airway mucosa. In theory, a reduced microvascular flow would decrease the rate of delivery of plasma to the venular sites of leakage and, thus, vasoconstriction might be a way to reduce the plasma exudation response. However, in the present study, budesonide was without antiexudative effects, indicating that any vascoconstrictor action would have been insufficient to reduce the extravasation process. Indeed, we have previously demonstrated that a strong vasoconstrictor, such as oxymetazoline, may not attenuate airways plasma exudation responses [15].

Mucosal exudation of plasma is also a feature of asthma [16, 17], and it is also inhibited in this disease by glucocorticoid treatment [4]. It is difficult to examine mechanisms of plasma exudation across the human tracheobronchial mucosa with great specificity: The concentration and distribution of challenge agents and lavage fluids on the mucosa may not be well-controlled, and it may be difficult to distinguish between lavages of the airway, where the asthma resides, and the large alveolar surface. The upper and the lower airways exhibit similar plasma exudation responses to inflammatory challenges [1], and in disease similar topical doses of glucocorticoids, or relatively less in the bronchi, are anti-exudative in the two airway domains [3, 4]. It is possible, therefore, that the present findings may also by valid for the tracheobronchial airways.

Mucosal exudation of plasma in asthma and rhinitis is a sign that inflammatory cell processes of the mucosa/submucosa are not trivial, but involve significant activation of an airway end-organ response [1]. The lack of direct antipermeability efficacy in human airways suggests that the antiexudative efficacy of topical glucocorticoids in airway disease reflects the ability of these drugs to suppress cellular mechanisms that are significantly involved in driving inflammatory airway processes.

In conclusion, we suggest that the vascular antipermeability effects of glucocorticoids demonstrated in animal airways may not be valid for clinically-employed drug doses in human airways. Hence, the antiexudative effect of glucocorticoids seen in inflammatory airway diseases may not be a direct effect on the postcapillary venular endothelial cells, but rather an effect on crucial cellular mechanisms that drive and perpetuate the inflammatory airway process.

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