Effects of inhaled histamine, methacholine and capsaicin on sputum levels of α_2 -macroglobulin

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Abstract

Background – Plasma exudation-derived proteins and peptides contribute significantly to inflammation in the airway mucosa in vivo. In the guinea pig trachea both histamine and the neurogenic stimulant capsaicin produce acute mucosal tissue distribution and luminal entry of bulk plasma, whereas cholinergic agonists fail to produce this effect. Of these agents, only histamine induces mucosal exudation of plasma in human nasal airways. The exudative effect of the above agents on human bronchi remains unknown.

Methods – The bronchial exudative responses to inhalation of histamine, methacholine, and capsaicin were examined in two groups of healthy volunteers. Sputum was induced on three occasions in each study group by inhalation of hypertonic saline (4.5%) given as an aerosol for 40 minutes using an ultrasonic nebuliser. The second and third occasions were preceded by histamine and capsaicin challenges in the first study group, and by histamine and methacholine challenges in the second study group. Histamine and methacholine were given in cumulative doses (total doses 3160 μ g, respectively) or until a 20% reduction in forced expiratory volume in one second (FEV₁) was achieved. Cumulative doses of capsaicin were inhaled until coughing prevented the subjects from drawing a full breath. Sputum levels of α_2 -macroglobulin (720 kDa) were measured as an index of mucosal exudation of bulk plasma.

Results – Histamine increased mean (SE) sputum levels of α_2 -macroglobulin from 2.72 (1.01) μ g/ml (95% confidence interval (CI) 0.49 to 4.94) to 18.38 (8.03) μ g/ml (95% CI 0.49 to 36.27) in the first group, and from 1.66 (0.84) μ g/ml (95% CI –0.18 to 3.49) to 9.43 (3.63) μ g/ml (95% CI 1.59 to 17.27) in the second group. In contrast, capsaicin evoked no exudation (sputum levels of α_2 -macroglobulin 1.21 (0.28) μ g/ml (95% CI 0.59 to 1.83)) and methacholine produced a minor increase in sputum levels of α_2 -macroglobulin (2.90 (0.92) μ g/ml (95% CI 0.90 to 4.89)).

Conclusions – These results indicate that histamine is a useful agent for studying bronchial exudative responsiveness in man and that exudative effects are only of marginal importance in the cough and

bronchoconstriction produced by capsaicin and methacholine.

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Keywords: histamine, methacholine, capsaicin, α_2 -macroglobulin, sputum.

Inhalation of either histamine or methacholine is widely used to assess bronchial hyperresponsiveness.^{1,2} These two agents contract tracheobronchial smooth muscle and it is largely this pharmacological action that causes airway obstruction. However, other actions are not common to both mediators – for example, histamine, being an inflammatory mediator, is a potent exudative agent in many tissues including animal airways³ and human nasal airways⁴ whereas methacholine and analogues are without this action or evoke only marginal increases in mucosal output of plasma proteins in vivo, even at high dosage levels.^{3,5}

Inhaled capsaicin causes cough in animals and man.^{6,7} In guinea pigs and rats, capsaicin also induces prompt entry of plasma (neurogenic exudative inflammation) into the airway lumen.⁸ Increased microvascular permeability, whether due to capsaicin or histamine, is best determined as luminal entry of bulk plasma; plasma extravasated in response to histamine will thus promptly and effectively be transmitted across the epithelial lining, even in airways with a healthy epithelium that has a normal tight junction as an absorption barrier.⁹ This swift and obligatory epithelial passage of bulk plasma may be important in airway defence reactions because the biologically active peptides and proteins contained in the plasma exudate can neutralise agents already on the mucosal surface before epithelial integrity is lost.¹⁰ The epithelial passage has practical research consequences; exudative indices contained in readily obtained airway surface liquids may adequately reflect increases in the permeability of the subepithelial microcirculation.⁹ Whereas capsaicin evokes luminal entry of bulk plasma in guinea pig airways,⁸ this response has been difficult to detect in human nasal airways.¹¹⁻¹³ Indeed, capsaicin given at dose levels which cause pain has not produced any detectable exudative response¹³ even in the allergic nasal airways that exhibit both neurogenic hyperresponsiveness (increased pain to topical capsaicin¹³) and microvascular exudative hyperresponsiveness (exaggerated exudative response to topical histamine¹⁴).

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The present study was undertaken because there is little information about exudative responses to inhaled mediators in human bronchi. Moreover, even if topical allergen challenge has indicated that there is a luminal entry of bulk plasma in both human bronchi^{15,16} and human nasal passages,^{16,17} there is no general acceptance of the concept that exudative properties of mediators will be expressed similarly in human nasal and bronchial mucosae. In this study we have thus tested the hypothesis that histamine is an exudative agent in human bronchi. We have also examined whether methacholine and capsaicin produce plasma exudation responses in the lower human airways. In order to obtain airway surface samples we have employed the technique of sputum induction by inhalation of hypertonic saline. As an index of luminal entry of bulk plasma we have analysed sputum levels of α_2 -macroglobulin which is one of the largest plasma proteins and which previously has been validated as a particularly useful index of plasma exudation responses in human airways.^{14,16}

Methods

STUDY DESIGN

Sputum was induced by inhalation of aerosolised hypertonic saline in two separate studies each performed on three study days. On the first day sputum induction was performed without any pharmacological challenge, on the second day sputum induction was preceded by inhalation of aerosolised histamine, and on the third day by inhalation of aerosolised capsaicin (first study) or methacholine (second study). The sputum concentration of α_2 -macroglobulin was measured as an index of mucosal exudation of bulk plasma. The washout time between the challenges was two weeks. The study was of an open design.

SUBJECTS

Study 1 comprised 11 healthy subjects (nine men) aged 22–30 years and study 2 comprised 14 separate healthy subjects (seven men) aged 22–25 years. The subjects had no history of allergy, asthma, or recent nasal and bronchial disease, and no history of recent drug treatment. They all had a baseline forced expiratory volume in one second (FEV₁) of more than 90% of the predicted FEV₁. The study was approved by the local ethics committee and informed consent was obtained.

ADMINISTRATION OF AEROSOLS

Bronchial challenges with histamine, capsaicin, and methacholine were carried out using a dosimetric aerosol delivery system based on an air jet nebuliser (Spira Electro 2, Respiratory Care Center, Hämeenlinna, Finland). Aerosol delivery was set to commence after inhalation of 0.1 litres and nebulisation then continued for one second. The volume of each inspiration was approximately 1.0 litre and the flow rate was approximately 0.5 l/s. The inhaled dose was varied by changing the concentration of

the nebulised solution as well as the number of inhaled breaths. Three concentrations were used for each agent. The maximum number of breaths for one dose was 12. Hypertonic saline was administered using an ultrasonic nebuliser (Aerosonic, DeVilbiss, Somerset, Pennsylvania, USA) which generates droplets with a mass median diameter of 1–5 μ m at a minimum nebulisation rate of 0.3 ml/min.

BRONCHIAL HISTAMINE AND METHACHOLINE CHALLENGES

Before the histamine and methacholine challenge series a baseline FEV₁ was recorded – that is, the highest FEV₁ recorded of three consecutive tests – using an electronic spirometer (Vitalograph-Compact II, Buckingham, UK). Thereafter, the subjects inhaled increasing doses of histamine or methacholine starting with an initial dose of 2 μ g. FEV₁ was recorded one and three minutes after each challenge. Challenges were continued until a cumulative dose of 3160 μ g had been given or until a 20% decrease in FEV₁ from baseline was achieved.

BRONCHIAL CAPSAICIN CHALLENGE

Before the capsaicin challenge series a baseline FEV₁ was recorded as described above. Thereafter, the subjects inhaled increasing doses of capsaicin starting with an initial dose of 1.5 ng. The number of coughs was monitored and FEV₁ was recorded one and three minutes after each challenge. Challenges were continued until a cumulative dose of 2410 ng had been given or until a 20% decrease in FEV₁ from baseline was achieved, or until the subjects were unable to take a full breath due to coughing.

SPUTUM INDUCTION

Sputum was induced by inhalation of hypertonic saline at control and at about five minutes after the histamine, methacholine, and capsaicin challenge tests. Aerosolised hypertonic saline (4.5%) was inhaled at resting ventilation rate for 40 minutes. Thereafter, the subject was instructed to rinse the mouth three times with 20 ml water, to clear the throat, and to cough sputum into a container. The quality of the sample was assessed by its macroscopic appearance on a Petri dish. The sample was put into a tube, without attempting to separate sputum from small amounts of any additional fluid, and frozen (–20°C) for later analysis of α_2 -macroglobulin.

ANALYSIS OF α_2 -MACROGLOBULIN

The sputum samples were processed by ultrasonication for 15 minutes and centrifugation at 32 000g for 15 minutes. This procedure was chosen in favour of mucolytic agents to avoid interference with the α_2 -macroglobulin analysis. The levels of α_2 -macroglobulin were measured using a radioimmunoassay sensitive to 7.8 ng/ml. Rabbit anti-human α_2 -macroglobulin (Dakopatts, Copenhagen, Denmark)

Table 1 Effects of inhalational histamine, capsaicin, and methacholine challenges on mean (SE) FEV₁ (%) recorded three minutes after challenge in study groups 1 and 2

Study group 1						Study group 2					
Histamine (µg)	FEV ₁ (%)	No. of subjects	Capsaicin (ng)	FEV ₁ (%)	No. of subjects	Histamine (µg)	FEV ₁ (%)	No. of subjects	Methacholine (µg)	FEV ₁ (%)	No. of subjects
2	96 (1)	11	1.5	99 (1)	11	2	99 (1)	14	2	98 (1)	14
10	97 (1)	11	7.6	99 (1)	11	10	100 (1)	14	10	98 (1)	14
40	98 (1)	11	31	100 (1)	11	40	99 (1)	14	40	97 (1)	14
160	95 (2)	11	122	99 (1)	10	160	91 (3)	14	160	95 (2)	14
460	86 (3)	11	351	101 (1)	5	460	77 (4)	13	460	93 (2)	13
1360	80 (3)	8	1037	100 (1)	5	1360	77 (4)	7	1360	87 (2)	13
3160	78 (4)	3	2410	101 (2)	4	3160	83 (0)	1	3160	86 (2)	10

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-assay and inter-assay coefficients of variation were 3.8–6.0% and 3.1–7.2%, respectively.

DATA ANALYSIS

Differences in sputum levels of α_2 -macroglobulin between control and challenge situations were analysed by Friedman test and, if statistical significance emerged, by Wilcoxon signed rank test. A *p* value of less than 0.05 was considered significant. Data are presented as mean (SE), with or without 95% confidence intervals (CI) as appropriate.

Results

The baseline FEV₁ recorded before the challenge and sputum induction series was reproducible in both study groups. The mean intra-individual coefficient of variation was 3.30 (0.8)% in the first group and 2.6 (0.5)% in the second group. Histamine produced dose dependent reductions in FEV₁ in both study groups. A mean final cumulative histamine dose of 1851 (376) µg was required to produce a 23 (2)% reduction in FEV₁ in the first group, and a mean dose of 996 (213) µg was required to produce a 28 (3)% reduction in FEV₁ in the second group (table 1). Similarly, in the second group methacholine produced a dose dependent reduction in FEV₁ with a mean final cumulative methacholine dose of 2528 (287) µg required to produce a 18.4 (2.6)% reduction in FEV₁ (table 1). In contrast, capsaicin did not significantly affect FEV₁ (table 1) but produced dose dependent cough finally so intense that

the subjects were unable to take a full breath. A mean final cumulative capsaicin dose of 1029 (340) ng produced this effect. The cumulative numbers of coughs associated with capsaicin challenges were 32 (9).

Sputum was obtained in nine out of 11 baseline inductions in the first study group and in all 14 baseline inductions in the second group. After challenge with histamine, capsaicin, and methacholine sputum was obtained at all inductions. Data points from subjects not producing baseline sputum samples have been excluded from the statistical evaluation, but if included they neither affect the outcome nor the results as presented below. The sputum volume recovered varied somewhat between subjects and between challenges, with recovered volumes after control saline, histamine, and capsaicin of 1.70 (0.30) g, 2.34 (0.37) g, and 1.65 (0.26) g, respectively, in the first study group and 1.75 (0.19) g, 3.04 (0.50) g, and 2.54 (0.46) g in the second study group. The sputum recovery was significantly increased after histamine in the second group compared with control saline (*p*<0.01) but otherwise the recovery was not significantly different in the sputum induction series.

Histamine produced significant mucosal exudation of α_2 -macroglobulin in both the first (*p*<0.05) and second study group (*p*<0.01) (table 2) with sputum levels of α_2 -macroglobulin increasing from 2.72 (1.01) µg/ml (95% CI 0.49 to 4.94) to 18.38 (8.03) µg/ml (95% CI 0.49 to 36.27) in the first study group, and from 1.66 (0.84) µg/ml (95% CI –0.18 to 3.49) to 9.43 (3.63) µg/ml (95% CI 1.59 to 17.27) in the second group. In contrast, capsaicin failed to produce mucosal exudation of α_2 -macroglobulin (1.21 (0.28) µg/ml (95% CI 0.59 to 1.83); table 2). Indeed, the total recovery of α_2 -macroglobulin (concentration of α_2 -macroglobulin multiplied by recovered volume of sputum) was significantly reduced by capsaicin challenge (*p*<0.05). Methacholine produced a minor but significant increase in the sputum levels of α_2 -macroglobulin (2.90 (0.92) µg/ml (95% CI 0.90 to 4.89), *p*<0.05; table 2).

Table 2 Mean (SE) sputum levels of α_2 -macroglobulin (µg/ml) at baseline and after challenge with histamine, capsaicin, and methacholine

Study group 1 (n=11)		Study group 2 (n=14)	
Challenge	α_2 -macroglobulin	Challenge	α_2 -macroglobulin
Control	2.72 (1.01)	Control	1.66 (0.84)
Histamine	18.38 (8.03)*	Histamine	9.43 (3.63)**
Capsaicin	1.21 (0.28)	Methacholine	2.90 (0.92)*

p*<0.05, *p*<0.01 difference between control and each challenge.

Discussion

This study, involving healthy subjects, has shown that histamine, in addition to bronchoconstriction, produces marked bronchial mucosal exudation of plasma (α_2 -macroglobulin). In contrast, methacholine produces bronchoconstriction but only marginally increases the

sputum levels of α_2 -macroglobulin. We have further shown that a dose of capsaicin that produces a very pronounced cough response fails to produce bronchial mucosal exudation of plasma. These observations provide some basic pharmacological information on the bronchial effects of airway challenge agents which are commonly employed in health and disease. They may also elucidate mechanisms in the human bronchial mucosa including whether or not neurogenic inflammation occurs in man.

Inhalation of hypertonic saline produces bronchoconstriction in patients with asthma whereas this response is not produced in healthy subjects.¹⁸ Another effect of inhalation of hypertonic saline in man is the induction of sputum production, particularly in patients with asthma, but also in healthy subjects. Whether this response represents a microvascular exudative effect has not been clarified. We have previously shown that nasal administration of hypertonic saline does not affect the baseline appearance of plasma proteins in nasal mucosal surface liquids, but that hypertonic saline may increase plasma exudation responses produced by histamine.¹⁹ If translatable to human bronchial airways, this finding suggests that hypertonic saline induced sputum production in combination with inhalation challenges with exudative inflammatory mediators, particularly histamine, may be used as an experimental tool in exploring human airway responses.

In the present study we took great care to rinse the oral, pharyngeal, and hypopharyngeal cavities after challenge and before attempting to cough sputum in order to minimise contamination of saliva.²⁰ This procedure increases the likelihood that the recovered α_2 -macroglobulin comes from the bronchial airways. The sputum levels of α_2 -macroglobulin have been expressed as the sample concentration, without any correction for sputum volume, thus reflecting as far as possible the concentration of α_2 -macroglobulin in airway mucosal surface liquids as they are after admixture with the inhaled saline and the additional "secretion" that is induced by deposits of hypertonic saline.

We have shown that inhaled histamine produces bronchial exudation of plasma in man. This exudative response was marked at a dose of histamine that produced about a 20% reduction in FEV₁. We have previously shown, by analysis of plasma proteins in nasal lavage fluids, that the mucosal output of α_2 -macroglobulin induced by histamine challenge can be taken to represent output of all plasma proteins.¹⁴ It has also been shown previously in animal airways that acute microvascular and epithelial passage of plasma induced by an inflammatory stimulus is a non-sieved process.³ It therefore appears likely that the levels of α_2 -macroglobulin recorded in the present study represent mucosal exudation of bulk plasma. It is evident from previous studies involving the human nose²¹ and the guinea pig tracheobronchial airways²² that, despite the marked increase in the outward (exudative) permeability of the airway epithelium, the mucosal absorption of luminal solutes in histamine ex-

posed airways may be unaffected. Hence, the histamine-induced microvascular and epithelial exudation of plasma in the present study cannot suggest any change in the absorption capacity of the bronchial mucosa.

Histamine may be an important mediator in airway inflammation. Particularly in acute allergic asthma, histamine has been found to contribute to the bronchial obstruction.²³ It has recently been shown that endobronchial allergen challenge is promptly followed by luminal entry of large plasma proteins including fibrinogen¹⁵ and α_2 -macroglobulin.¹⁶ The present data, although obtained in healthy subjects, suggest that histamine may be partly responsible for the biologically active molecular milieu of plasma-derived proteins and peptides in the allergic bronchi.

Capsaicin had no effect on bronchial exudation even when given in a dose that produced a strong cough response in healthy subjects. This indicates a high selectivity in action and supports the use of capsaicin as a specific test agent for the assessment of sensory cough responsiveness in human bronchi.^{6,7} The present observations with capsaicin also suggest that neurogenic inflammatory exudation may not occur in human bronchi. In view of the previous data on human nasal airways where capsaicin has failed to produce plasma exudation in allergic rhinitis even in the presence of a neurogenic hyperresponsiveness¹³ and a microvascular exudative hyperresponsiveness,¹⁴ this lack of exudation in the human airway should come as no surprise. Although our data argue strongly against the validity of the neurogenic inflammation hypothesis, we do not question the view that the human airway mucosa may hold tachykinergic sensory nerves and that peptide mediators from these nerves exert important physiological effects.²⁴⁻²⁶

The present results with methacholine inhalation are consistent with observations in the human nasal and the guinea pig tracheobronchial airways where even very high dose challenges with agent merely produce a slight increase in the levels of plasma proteins in the mucosal surface liquids.^{3,5} The slight (1.7-fold) increase in sputum levels of α_2 -macroglobulin seen after methacholine challenge, although statistically significant, needs to be confirmed since there is currently no known mechanism by which muscarinic agents may increase microvascular permeability. In contrast, methacholine and histamine are known to have about the same potency as constrictors of human bronchial smooth muscle.²⁷ The difference between histamine and methacholine in their effects on bronchial plasma exudation would favour the use of the latter agent as a more specific bronchoconstrictor by the inhaled route in man.

In studies using histamine as a topical challenge agent a microvascular exudative hyperresponsiveness has been reported in allergic rhinitis¹⁴ and common cold.²⁸ Hence, it would be of interest to explore bronchial exudative responsiveness in health and disease and the present method (involving histamine or another acute vascular permeability agent) may be

used for this purpose. The flooding of mucosal tissues with the potent binding proteins of plasma and the movement of this plasma exudate into the airway lumen may affect the fate of inflammatory cell products. Accordingly, in patients with ongoing allergic rhinitis we have found that simple histamine challenges move cell products such as interleukin 6, which is located in the allergic mucosal tissue, into the airway lumen.²⁹ It therefore appears possible to use histamine challenge as an experimental aid to improve the recovery of inflammatory cell products by lavage and sputum induction techniques. It should be of particular interest to examine the effect of histamine on the entry of cytokines into the lumen of the airway since these molecules are tightly bound and are carried by the α_2 -macroglobulin³⁰ that was shown to increase several fold in this study.

We conclude that mucosal exudation of plasma can be monitored in human bronchial airways by measurement of α_2 -macroglobulin in induced sputum. The known mechanisms of microvascular and epithelial exudation make it unlikely that a challenge could have produced extravasation of plasma into the lamina propria without incurring an increase in luminal levels of plasma proteins that could be sampled by sputum induction. In agreement with previous observations in guinea pig tracheal and human nasal airways, histamine produces mucosal exudation of bulk plasma in human bronchial airways. In contrast, methacholine had only a slight effect and capsaicin failed completely to produce exudative inflammation in human bronchial airways. The latter observation suggests that the concept of neurogenic inflammation may not translate to human bronchi in vivo. Taken together, the present data suggest that histamine may be a useful exudative challenge agent in human bronchi and support the pharmacological selectivity of methacholine and capsaicin as bronchoconstrictor and cough inducing agents, respectively.

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