

Effects of formoterol on histamine induced plasma exudation in induced sputum from normal subjects

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Abstract

Background—A number of studies have shown that β_2 agonists, including formoterol, inhibit plasma exudation induced by the inflammatory stimulus in animal airways. Whether clinical doses of β_2 agonists inhibit plasma exudation in human bronchial airways is unknown.

Methods—In order to explore the microvascular permeability and its potential inhibition by β_2 agonists in human bronchial airways a dual induction method was developed: plasma exudation induced by histamine inhalation followed by sputum induction by hypertonic saline (4.5%) inhalation. Sixteen healthy subjects received formoterol (18 μ g) in a placebo controlled, double blind, crossover study. Sputum was induced on five occasions: once at baseline and four times after histamine challenge (30 minutes and eight hours after both formoterol and placebo treatments). Sputum levels of α_1 -macroglobulin were determined to indicate microvascular-epithelial exudation of bulk plasma.

Results—Histamine induced plasma exudation 30 minutes after placebo was considerably greater than at baseline (median difference 11.3 μ g/ml (95% confidence interval 0.9 to 90.0)). At 30 minutes after formoterol the effect of histamine was reduced by 5.1 (0.9 to 61.9) μ g/ml compared with placebo. At eight hours histamine produced less exudation and inhibition by formoterol was not demonstrated.

Conclusion—This study shows for the first time an anti-exudative effect of a β_2 agonist in healthy human bronchial airways. Through its physical and biological effects, plasma exudation is of multipotential pathogenic importance in asthma. If the present findings translate to disease conditions, it suggests that an anti-exudative effect may contribute to the anti-asthmatic activity of formoterol.

(Thorax 1998;53:1010–1013)

Keywords: β_2 agonists; induced sputum; plasma exudation

way lumen are features of airway inflammation in asthma. Potentially, this process has pathogenic properties¹: the extravasated plasma contains potent adhesive and leucocyte activating proteins such as fibrinogen and fibronectin and inflammatory peptides including bradykinins and complement fragments. In addition, physical properties of the plasma exudate may affect the airway wall (oedema) and lumen (exudate-mucus plugs). Hence, extravasation and exudation of plasma may be important for the in vivo mucosal biology and the hyperresponsiveness of asthmatic bronchi. It has also been suggested that anti-exudative activity may be a desirable drug effect in the treatment of asthma.²

During the 1970s the vascular anti-permeability effect of β_2 agonists was clearly demonstrated.² Numerous groups have shown that β_2 agonists have the capacity to reduce extravasation of plasma in animal airways induced by the inflammatory stimulus^{3–8} and its further exudation into the airway lumen.^{9–10} The anti-exudative effect of inhaled formoterol showed, for the first time in animal airways, that it has a long duration of action.¹⁰ Although a vascular anti-permeability effect of formoterol¹⁰ has been confirmed by different authors,^{4–8} there is as yet no information regarding this potential property in human airways. Indeed, we know of no study that has shown the effects of any β_2 agonist on plasma exudation in human bronchi. In studies on human nasal airways high doses of terbutaline inhibited allergen challenge induced plasma exudation,¹¹ whereas salmeterol failed to inhibit significantly the increased nasal lavage fluid levels of albumin found after challenge with allergen or histamine.¹²

The exudative responsiveness of the subepithelial microcirculation of human bronchi may be specifically examined using histamine challenges followed by sputum induction with hypertonic saline.¹³ In the present study we have examined whether or not formoterol, given at a therapeutic dose level, has the capacity to reduce bronchial exudation of plasma in healthy human subjects.

Methods

STUDY DESIGN

Healthy subjects received formoterol in a randomised, placebo controlled, double blind,

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Received 26 May 1998
Returned to author
27 July 1998
Revised manuscript received
14 August 1998
Accepted for publication
3 September 1998

Extravasation, distribution in the lamina propria, and entry of bulk plasma into the air-

crossover design. Sputum was induced on four occasions after histamine challenge (30 minutes and eight hours after both formoterol and placebo treatments). Furthermore, baseline sputum was induced on a separate occasion. Sputum levels of α_2 -macroglobulin were determined to indicate airway luminal exudation of plasma. The washout time between each test was two weeks.

SUBJECTS

Sixteen healthy subjects aged 24–31 years participated in the study. Inclusion criteria were age of 18 years or more, a negative skin prick test to seasonal and perennial allergens, and normal spirometric values. Subjects with a history of chronic bronchial disease including asthma, a history of recent respiratory tract infection, a history of chronic nasal disease, those undergoing drug treatment, smokers, and pregnant women were excluded. No medication other than the study drug was allowed during the study period.

STUDY DRUG

Placebo and formoterol fumarate (Oxis[®], Turbuhaler[®], 12 μ g metered dose, 9 μ g delivered dose; Astra Draco, Lund, Sweden) were given as two consecutive inhalations (total dose 18 μ g). The patients were given allocation numbers and the order of treatment proceeded according to a randomisation scheme of the allocation numbers prepared in blocks of four. Blindness was maintained by identical appearance of active and placebo preparations.

BRONCHIAL HISTAMINE CHALLENGE

Bronchial challenges with histamine were given 30 minutes and eight hours after administration of placebo and formoterol. The challenges 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 was then continued for 1.0 seconds. The volume of each inspiration was approximately 1.0 litres and the flow rate approximately 0.5 l/s. The inhaled dose was varied by changes in the concentration of the nebulised solution as well as the number of inhaled breaths.¹³ Three different histamine concentrations were used: 0.4, 6.0, and 30.0 mg/ml. The maximum number of breaths per dose was 12. The histamine challenge series resulted in the following cumulative doses: 2, 10, 40, 160, 460, and 1360 μ g.

Before each histamine challenge series a baseline forced expiratory volume in one second (FEV₁) was recorded—that is the maximum FEV₁ recorded of three consecutive tests—using an electronic spirometer (Vitalograph Compact II, Buckingham, UK). Thereafter, the subjects inhaled increasing doses of histamine starting with an initial dose of 2 μ g. FEV₁ was recorded one and three minutes after each challenge, and challenges were continued until a cumulative dose of 1360 μ g had been given or until a 20% decrease in FEV₁ from the baseline value was achieved. The reduction in

FEV₁ was expressed as the percentage change from baseline at that particular visit (FEV₁ (% baseline)). The same cumulative dose of histamine was given at the second, third, and fourth challenge—that is, after both placebo and formoterol treatment. (The cumulative dose of histamine was chosen as the dose likely to be tolerated when the subjects also received placebo treatment.¹³)

SPUTUM INDUCTION

Sputum was induced by inhalation of hypertonic saline about five minutes after administration of each histamine challenge series as well as at baseline, prior to histamine challenge. Aerosolised hypertonic saline (4.5%) was inhaled at resting ventilation rate for 40 minutes using an ultrasonic nebuliser (Aerosonic, DeVilbiss Health Care, Somerset, Pennsylvania, USA).¹³ 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 the 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 (15 minutes) and centrifugation at 32 000g for 15 minutes. 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) 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). As reported previously,¹³ the intra- and inter-assay coefficients of variation are 3.8–6.0% and 3.1–7.2%, respectively.

ANALYSIS OF DATA

Differences in sputum levels of α_2 -macroglobulin between control and challenge, and between placebo and formoterol, were analysed by the Friedman test when appropriate and, if statistical significance emerged, by the Wilcoxon signed rank test. A p value of less than 0.05 was considered significant. Furthermore, median differences with 95% confidence intervals (CI) were calculated for comparisons between baseline and placebo as well as between placebo and formoterol. Data are presented as mean (SE) values.

Results

The sputum induction procedure was successful in 15 of 16 inductions carried out at baseline and in 63 of 64 inductions carried out

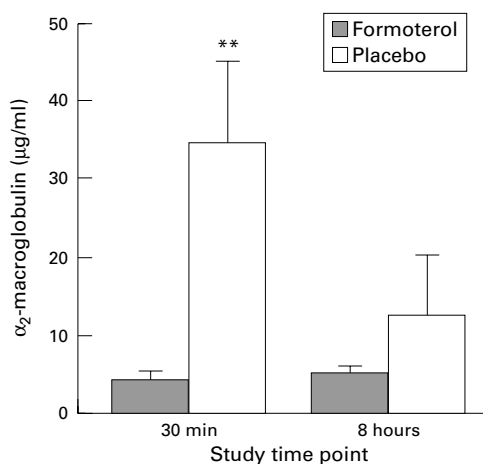


Figure 1 Mean (SE) effects of placebo and formoterol on histamine induced bronchial luminal exudation of plasma (sputum α_2 -macroglobulin) 30 minutes and eight hours after treatment. ** $p < 0.01$.

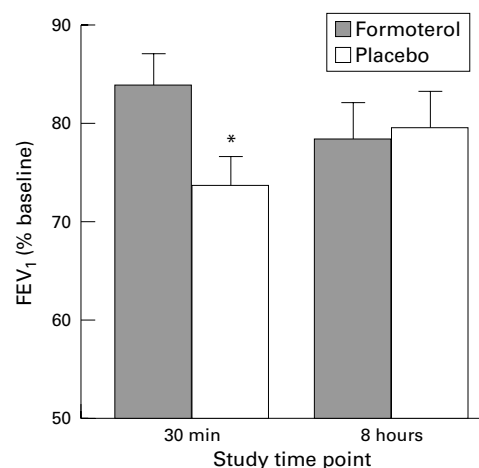


Figure 2 Mean (SE) effects of placebo and formoterol on histamine induced reductions in forced expiratory volume in one second (FEV₁) 30 minutes and eight hours after treatment. * $p < 0.05$.

after histamine challenge. The sputum recovery was 1.9 (0.4) g at baseline and 1.5 (0.3) g and 1.3 (0.3) g at 30 minutes and eight hours after histamine challenge following placebo treatment. The recovery was 0.8 (0.2) g ($p < 0.01$, median difference 0.6 g (95% CI 0.1 to 0.9) compared with placebo) and 0.8 (0.1) g ($p = 0.2$, median difference 0.3 g (95% CI 0.0 to 0.5) compared with placebo) at 30 minutes and eight hours after histamine challenge following formoterol treatment. The concentration of α_2 -macroglobulin in sputum obtained at baseline induction was 1.89 (0.41) $\mu\text{g/ml}$. Sputum induction carried out at baseline was associated with a minor but statistically significant decrease in FEV₁ ($p < 0.001$) with a median difference of 0.2 litres (95% CI 0.1 to 0.2).

In subjects receiving placebo the first histamine challenge (30 minutes after treatment) caused a marked increase in mucosal output of α_2 -macroglobulin ($p < 0.01$ compared with baseline). The median difference was 11.3 $\mu\text{g/ml}$ (95% CI 0.9 to 90.0). This response was markedly attenuated by formoterol treatment ($p < 0.01$ compared with placebo; fig 1); the response to histamine was reduced by a median 5.1 $\mu\text{g/ml}$ (95% CI 0.9 to 61.9). After histamine challenge at eight hours after treatment (when the induced exudation was less than at 30 minutes with placebo treatment; $p < 0.05$) there was no significant difference in bronchial mucosal output of α_2 -macroglobulin between placebo and formoterol ($p = 0.9$; fig 1), the median difference being 0.2 $\mu\text{g/ml}$ (95% CI -4.4 to 3.5).

After placebo the first histamine challenge (30 minutes after treatment) reduced FEV₁ from the baseline value by a median 0.8 litres (95% CI 0.7 to 1.0 l); $p < 0.001$. This response was attenuated by formoterol ($p < 0.05$; fig 2); the median difference in the response to histamine between placebo and formoterol was 0.4 litres (95% CI -0.2 to 0.7). After the second histamine challenge series eight hours after treatment the response in the placebo group was somewhat less pronounced than after 30 minutes and there were no significant

differences in histamine induced reduction in FEV₁ between subjects receiving placebo and those receiving formoterol ($p = 0.7$; median difference 0.1 litres (95% CI 0.0 to 0.2); fig 2).

Discussion

This study, involving healthy subjects, has shown that formoterol is a potent inhibitor of histamine induced human bronchial exudation of bulk plasma. Reducing plasma protein extravasation may be important together with bronchodilatation in the treatment of asthma with β_2 agonists.

The present challenge followed by sputum induction revealed a marked acute bronchial exudation of α_2 -macroglobulin by histamine. These data agree with previous results obtained with this "dual induction" method—that is, induced exudation and induced sputum.¹³ Studies involving the human nose indicate that histamine induced exudation of α_2 -macroglobulin reflects luminal entry of non-sieved plasma, and that this nature of the response may be independent of the concentration of histamine.¹ Animal airway experiments have shown that the extravasated plasma distributes in the lamina propria, moves up between and all around epithelial cells in the challenge area, and gently enters the airway lumen as a result of the plasticity of the apical epithelial cell junctions. Hence, it is possible to assess microvascular permeability changes by merely analysing luminal airway samples as in the present study. Indeed, the paraepithelial clearance of large plasma protein molecules occurs without injury and without compromising the normal function of the epithelial lining as absorption barrier. The pluripotent plasma derived molecules may thus be present and may also contribute to the mucosal *in vivo* biology in non-injurious airway inflammatory conditions.¹

By demonstrating the anti-exudative efficacy of a clinically used dose of formoterol, this study has added weight to early suggestions² that a vascular anti-permeability effect may be a facet of the treatment of asthma with β_2 agonists. An anti-exudative action could, in part,

explain the anti-asthma efficacy of β_2 agonists that goes beyond bronchodilatation and that may involve reduced numbers of exacerbations of the disease.¹⁴ Although animal data suggest that formoterol is also an effective vascular anti-permeability agent in inflamed airways,⁶ the present findings need to be confirmed in asthmatic bronchi. Furthermore, it is important to explore the possibility of tachyphylaxis to the anti-exudative action. In the present study we failed to show an anti-exudative effect of formoterol eight hours after inhalation. However, this may be due to the reduced response to the second histamine challenge (fig 1). A reduced plasma exudative effect has also been observed in human nasal mucosa when repeatedly challenged with a low dose of histamine.¹⁵

In conclusion, the present data indicate that anti-exudative activity is a significant component of the human bronchial pharmacology of a clinical dose of inhaled formoterol.

This study was supported by the Swedish Medical Research Council (projects 8308 and 10841), the Vårdal Foundation, the Swedish Association against Asthma and Allergy, and Astra Draco, Sweden. We thank Lena Glans for laboratory assistance.

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