Failure of frusemide to increase production of prostaglandin E₂ in human nasal mucosa in vivo

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Abstract

Background It has been suggested that inhaled frusemide protects subjects with asthma against bronchoconstriction by enhancing the synthesis of prostaglandin E₂ (PGE₂). To evaluate this hypothesis the effect of frusemide on PGE₂ production from nasal mucosa was studied.

Methods Two main arachidonic acid metabolites produced by epithelial cells, PGE₂ and 15-hydroxy 5,8,11,13-eicosatetraenoic acid (15-HETE), were measured by radioimmunoassay in nasal secretions obtained by nasal lavages with saline. Eleven healthy volunteers were randomly assigned to two study days, one week apart, in a double blind crossover study. Nasal instillation with three increasing doses of frusemide (5, 10, and 20 mg) or placebo was carried out at intervals of 15 minutes. Nasal lavages were performed immediately before nasal instillations and 15, 30, and 60 minutes after the last instillation.

Results Baseline concentrations of 15-HETE were at least six times higher than PGE₂. No differences between frusemide and placebo were detected either on PGE₂ or 15-HETE release.

Conclusions The findings do not support the hypothesis that the antiasthmatic effect of frusemide may be due to increased synthesis of PGE₂ or release in the respiratory mucosa.

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effect.⁶ PGE₂ is a potent immunomodulator and a moderate bronchodilator. It has been proposed that frusemide might prevent bronchoconstriction by enhancing PGE₂ synthesis.⁷ Furthermore, it is interesting to note that the protective effect of frusemide on bronchoconstriction is similar to the effect of disodium cromoglycate, a drug that also prevents asthma attacks induced by exposure to allergen, exercise, and distilled water.⁸⁻¹⁰ In a recent study, the authors reported that disodium cromoglycate effectively attenuates the release of 15-hydroxy,5,8,11,13-eicosatetraenoic acid (15-HETE) after allergen provocation of the nasal mucosa.¹¹ This study also reported a decrease in basal 15-HETE release from nasal mucosa by disodium cromoglycate in both rhinitic and healthy subjects.¹¹ As comparable results for both frusemide and cromoglycate have been found, we considered that frusemide, like disodium cromoglycate, might protect against inflammatory reactions by inhibiting the release of 15-HETE.

Frusemide increases diuresis in healthy subjects and it is therefore reasonable to speculate that it might also exert its effects on the release of arachidonic acid metabolites in the upper airway of healthy subjects. Because an accurate knowledge of the changes induced by frusemide in the production of chemical mediators in healthy subjects may help to better understand the mechanisms involved in its protective effect on bronchospastic reactions, we have carried out a study to evaluate the effects of frusemide on the release of the main eicosanoids (15-HETE and PGE₂) produced by the respiratory epithelia.

Subjects and methods

POPULATION

Eleven healthy volunteers (eight men and three women) aged 35 (SD 5) years participated in the study. They were recruited from the staff of our institution. The criteria for inclusion were current good health and absence of any treatment at the time of the study. None of the volunteers had had a respiratory infection for at least three weeks and they did not take any anti-inflammatory medication during the week before the study. All subjects gave informed consent to participate in the study, which was approved by the research committee of our institution.

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Frusemide has been reported to inhibit the bronchoconstrictor responses to various stimuli such as exposure to allergen, distilled water, adenosine, and sodium metabisulphite, and exercise.¹⁻⁵ The mechanisms involved in this non-specific, protective effect of frusemide are not clear. As bronchospastic responses induced by all these stimuli have been linked to an excessive liberation of bronchoconstrictor agents from inflammatory cells, it has been suggested that frusemide may exert its protective effect by modifying the release of chemical mediators. Frusemide, a loop diuretic, increases the production of prostaglandin E₂ (PGE₂) in the kidney and this action has been related to its diuretic

NASAL LAVAGE

Nasal lavages were carried out according to the method described by Naclerio and coworkers.¹² Briefly, with the head tilted backward, 8 ml of normal saline (0.9% NaCl) was instilled into the nose (4 ml in each nasal cavity) while the subject refrained from breathing and swallowing. After about 10 seconds, the subjects expelled the mixture of saline and nasal secretions into polypropylene tubes, which were stored at -70°C until extraction. Nasal lavage recoveries in the present study were 5.5 (SD 0.1) ml (70 (SD 1)%).

RADIOIMMUNOASSAY OF ARACHIDONIC ACID METABOLITES

PGE₂

Extraction of PGE₂ from nasal lavages was performed according to a method previously described.¹³⁻¹⁶ Nasal lavages were centrifuged at 20 000 g at 4°C for 15 minutes. Aliquots of supernatant fluid (0.5 ml) were acidified at pH 3 and processed through C18 cartridge columns (Baker, Phillipsburgs, NJ, USA). After washing with 10 ml acidified water and 20 ml petroleum ether, prostaglandins were eluted with 8 ml methyl formate, which was evaporated in a vacuum (Savant, Hicksville, NY, USA). Dried residues were redissolved in Tris buffer and PGE₂ was determined by radioimmunoassay (RIA) as previously described with antiserum provided by the Institute Pasteur (Marnes la Coquette, France). Standard curves were generated from 1.9 to 500 pg/tube and samples were diluted 1:2.08. The recovery of radiolabelled ³H-PGE₂ used as an internal standard of the assay was 86.4%.

The precision of the RIA method has been defined by calculating the intra-assay coefficient of variation (CV) for 10 repetitive assays of three nasal lavage samples. The table shows the results. The reliability of the procedure is supported by the 0.999 correlation coefficient calculated for nasal lavage samples supplemented with authentic PGE₂ (0, 5, 10, 20, 40, and 80 pg) and the corresponding values obtained by RIA (n = 5).

15-HETE

The extraction of 15-HETE from nasal lavages was performed according to a method previously described.^{14,17} Supernatant aliquots (2 ml) were processed without pH modification through C18 Baker cartridge columns. After a wash with 10 ml of water, 15-HETE was eluted with 5 ml methanol:water (9:1).

Mean (SD) response of repetitive RIA determinations (n=10) of three different nasal lavage samples

PGE ₂	Response	CV (%)
Supplemented sample*	42.5 (1.5)	3.5
Acetylsalicylic acid inhibited†	5.3 (0.5)	9.8

*Diluted 1:3 to fit the response to the most precise maximum slope part of the RIA standard curve.
†Sample from a subject who had ingested 500 mg of the prostaglandin synthesis inhibitor acetylsalicylic acid.

The eluent was then evaporated to dryness in a vacuum. Residues were redissolved in RIA buffer and 15-HETE was determined with RIA kits provided by Amersham. Antibodies were highly specific for 15-HETE. Standard curves were generated from 16 to 625 pg/tube and samples diluted 1:3.7. The recovery of radiolabelled ³H-15-HETE used as an internal standard was 97.1%.

As shown by the parallelism of 15-HETE standards and nasal lavage samples assayed with serial dilutions (1:4, 1:16, 1:32, 1:64 and 1:128), these tests provide an indirect measure of the RIA specificity because sample immunoreactivity behaves in a similar manner to the standard.¹⁷

Study design

A double blind crossover study design was used. Subjects were randomly divided into two study days one week apart. After a basal nasal lavage (time 0), three increasing doses of frusemide (5, 10, and 20 mg) were nebulised into the right nasal cavity by a hand driven nebuliser at 0, 15, and 30 minutes. Nasal lavages were carried out 15 minutes after each dose of frusemide and 30 minutes after the last dose was administered (figs 1 and 2). Equal volumes of placebo solution (0.5, 1, and 2 ml of 0.9% NaCl) were instilled in the right nasal cavity in the same manner as frusemide. As the frusemide solution used in our study had a pH of 9, the placebo solution (initial pH 7.3) was adjusted to pH 9 by the addition of 0.1 M NaOH.

Analysis

Results are presented as mean (SE) values in pg/ml. The statistical evaluation was performed on a microcomputer (Macintosh II, Apple, Cupertino, CA, USA) with a statistical software package (Statview 512+, Brainpower Inc, Calabasas, CA, USA). Student's *t* test for paired sample analysis was used for statistical comparison between frusemide and placebo effects. Values <0.05 were considered statistically significant.

Figure 1 Time course of mean PGE₂ release in nasal secretions. Concentrations of PGE₂ decreased after nasal administration of three increasing doses of frusemide (5, 10, and 20 mg; arrows) (solid line) or placebo (broken line). No significant differences were found between frusemide and placebo treated subjects (n=11).

Figure 2 Time course of mean 15-HETE release in nasal secretions. Concentrations of 15-HETE decreased after nasal administration of three increasing doses of frusemide (5, 10, and 20 mg; arrows) (solid line) or placebo (broken line). No significant differences were found between frusemide and placebo treated subjects ($n = 11$).

Results

Basal 15-HETE production, 814 (270) pg/ml on the placebo day and 869 (354) pg/ml on the frusemide day ($n=11$) was at least six times higher than basal PGE_2 production, 125.5 (21) pg/ml and 130 (17) pg/ml ($n=11$). Basal concentrations of 15-HETE and PGE_2 were not statistically different between placebo and frusemide days. The procedure was well tolerated and instillation of placebo (saline) and frusemide only caused minor symptoms.

PGE_2

Figure 1 shows the effect of frusemide *v* placebo on PGE_2 production by human nasal mucosa *in vivo*. After nasal lavages, PGE_2 basal concentration in nasal secretions showed a progressive decrease starting at 30 minutes (115 (16) pg/ml for placebo and 106 (19) pg/ml for frusemide), reaching the lowest values (54 (9) pg/ml and 55 (9.6) pg/ml) at 60 minutes. No significant changes in PGE_2 production were found between placebo and frusemide instillation.

15-HETE

Figure 2 shows the effect of frusemide on 15-HETE production. As for PGE_2 , no differences in the production of this arachidonic acid metabolite were found in basal values between the placebo day and the frusemide day. The production of 15-HETE decreased progressively starting at 15 minutes (777 (291) pg/ml for placebo and 592 (170) pg/ml for frusemide) and reached the lowest concentration at 60 minutes (328 (79) pg/ml and 330 (49) pg/ml respectively). There were no statistical differences between placebo and frusemide instillation.

Discussion

The bronchoconstrictor response in asthma depends on a complex interaction between inflammatory cells and their products, epithelial cells, neural pathways, and airway smooth

muscle. Frusemide may prevent asthmatic responses by interfering with one or more of the mechanisms involved in asthma.^{1 2 4 18} For instance, Anderson and coworkers¹⁹ have reported that frusemide may prevent the release of histamine and leukotrienes from human lung tissue passively sensitised to *Dermatophagoides pteronyssinus*. The relevance of these *in vitro* findings to *in vivo* allergen induced asthma has yet to be evaluated.

Frusemide is an inhibitor of sodium-potassium chloride cotransport located in the basolateral cell membrane.²⁰ Mucosal application of frusemide, however, had no effect on cotransport function.^{4 20} Another diuretic, amiloride, shows a greater effect than frusemide on epithelial ion flux but it does not prevent bronchoconstriction.⁴ Altogether, these findings suggest that inhibition of ion transport cannot account for the protective effect of frusemide. On the other hand, studies have shown that the antiasthmatic effect of frusemide is unlikely to be due to a direct effect on smooth muscle contraction.¹⁸ The protective effect of frusemide on cough induced by low chloride (Cl^-) solution indicates that it might protect from bronchospasm by acting on airway sensory nerves but confirmation of this hypothesis requires further studies.⁴

Inhaled PGE_2 , like inhaled frusemide, may inhibit bronchoconstrictor response to allergen, exercise, and metabisulphite.⁷ These findings, together with the reported stimulatory effect of frusemide on PGE_2 release,⁶ suggest that frusemide might prevent bronchospasm by enhancing PGE_2 production in the respiratory mucosa. The results of the present study, however, do not support this hypothesis.

15-HETE and other metabolites formed from arachidonic acid via 15-lipoxygenase have the capacity to promote inflammatory and hypersecretory responses in the airways.^{21 22} As 15-HETE can also modulate the generation of lipoxygenase products²³ it has been suggested that 15-HETE might also participate in the regulation of inflammatory responses in the upper airway. The recently described effect of disodium cromoglycate on 15-HETE production in the nose of subjects with rhinitis⁹ prompted us to investigate the effect of frusemide on the release of this arachidonic acid metabolite. Our study, however, could not find any effect of frusemide on 15-HETE production.

Different studies have shown that nasal lavages may be used to detect either an increase or decrease in the production of arachidonic acid metabolites.^{12 13} Because almost all studies with frusemide have been carried out in bronchial asthma, it might be argued that the results obtained with nasal lavages cannot rule out the possible implication of an increased production of PGE_2 at the bronchial level. Although the close similarities between nasal and bronchial mucosa, as far as arachidonic acid metabolite production is concerned,²⁴ suggest that the results obtained in the nose might be extrapolated to

the bronchi, a comparison study is necessary to confirm this.

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