The effect of inhaled frusemide on airway sensitivity to inhaled 4.5% sodium chloride aerosol in asthmatic subjects

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Abstract

Background Frusemide inhaled by asthmatic subjects before a variety of indirect bronchial challenges inhibits the airway response to these challenges. Since inhalation of hyperosmolar saline is an indirect bronchial challenge, the effect of inhaled frusemide and its vehicle on airway sensitivity to a 4.5% sodium chloride (NaCl) aerosol challenge was investigated.

Methods Eleven asthmatic subjects (five females, six males) who had a 20% fall in forced expiratory volume in one second after 4.5% NaCl challenge were enrolled in this double blind controlled crossover trial. Sensitivity was measured as the dose of aerosol required to provoke a 20% fall in FEV₁. Frusemide (33.2 mg) or its vehicle was delivered through a Fisoneb ultrasonic nebuliser and inhaled 10 minutes before challenge with 4.5% NaCl. A Mistogen ultrasonic nebuliser was used to generate the 4.5% NaCl aerosol and FEV₁ was measured before and one minute after each challenge period of 0.5, one, two, four, eight, eight and eight minutes. The doubling dose difference for PD₂₀ was calculated.

Results Frusemide or vehicle had no effect on baseline lung function. The geometric mean PD₂₀ after vehicle was 1.3 ml with a 95% confidence interval of 0.7-2.3 and after frusemide was 8.2 ml with a 95% confidence interval of 4.7-14.1. This represented a 2.6 doubling dose increase in PD₂₀ after frusemide inhalation. In five of the 11 subjects an increase from baseline FEV₁ occurred after exposure to 4.5% NaCl challenge in the presence of frusemide. This transient bronchodilatation may be caused by the release of prostaglandin E₂.

Conclusion Inhalation of frusemide is very effective in delaying airway narrowing induced by an aerosol of 4.5% NaCl in asthmatic subjects.

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Frusemide when given by inhalation to asthmatic subjects inhibits the airway narrowing provoked by exercise^{1,2} and hyper-

ventilation.^{3,4} It also inhibits the airway response to distilled water,^{5,6} allergen,⁷ metabisulphite,⁸ and adenosine 5'-monophosphate.⁹ Its effect on challenge with hyperosmolar saline has been reported only in abstract form.¹⁰

The precise mechanism by which frusemide inhibits the airway responses to this wide variety of provoking stimuli is still unknown. Its inhibitory effect on these challenges suggest that one possible mode of action may be in preventing release of mediators from mast cells. The in vitro finding that frusemide is effective in inhibiting the release of histamine and leukotrienes from passively sensitised human lung tissue in response to an allergen challenge supports this suggestion.¹¹

Hypertonic saline when inhaled by asthmatic subjects is thought to cause airway narrowing by releasing histamine from mast cells in the airways in response to an increase in airway osmolarity.¹²⁻¹⁶ The antihistamines terfenadine (given orally) and clemastine (given as an aerosol) are effective in reducing the airway narrowing provoked by an increase in airway osmolarity.¹⁷⁻¹⁹ As frusemide inhibits the release of histamine and other mast cell mediators it would be expected that frusemide would also inhibit airway narrowing when inhaled before a hypertonic saline challenge.

The purpose of this study was to compare the effects of frusemide given as an aerosol with its vehicle on airway responsiveness to 4.5% NaCl aerosol in a group of asthmatic subjects sensitive to increases in airway osmolarity.

Methods

SUBJECTS

Eleven clinically stable asthmatic subjects (five females, six males) ranging in age from 19 to 41 years with a mean age of 24.1 years attended the Respiratory Investigation Unit on three occasions over a period of 45 days. The subjects studied had a 20% fall in forced expiratory volume in one second (PD₂₀ FEV₁) after inhaling less than 18.0 ml of 4.5% NaCl. Anthropometric details, regular asthma medications, mean prechallenge percentage predicted FEV₁ values for the control

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Anthropometric details, mean prechallenge values for forced expiratory volume in one second (FEV₁) expressed as a percentage of the predicted value, provoking dose of 4.5% NaCl that caused a 20% fall in FEV₁ on the control day (PD₂₀ FEV₁), regular medications, and daily dose of inhaled beclomethasone dipropionate (values expressed as the geometric mean with 95% confidence interval)

Subject	Age (years)	Sex	FEV ₁ (% predicted)	PD ₂₀ FEV ₁ on control day (ml)	Regular medications	Daily dose of BDP (µg)
1	26	M	62.7	0.91	S	—
2	22	M	89.4	0.80	S	—
3	20	F	95.5	6.80	Ter, BDP	1000
4	30	F	79.0	2.93	S, I, BDP	1500
5	24	F	113.1	3.80	S, T, BDP	2000
6	20	F	97.6	1.49	S, BDP	2000
7	20	M	89.2	6.92	S	—
8	22	F	90.5	2.29	S, BDP	1000
9	19	M	121.8	3.17	S	—
10	41	M	72.8	0.89	F	—
11	21	M	87.8	0.47	S, BDP	1500
Mean (SD)	24.1 (6.5)		90.9 (16.7)	1.97 (95% CI 1.16–3.35)		

S—salbutamol; Ter—terbutaline; F—fenoterol; BDP—beclomethasone dipropionate; I—ipratropium bromide; T—theophylline

day and PD₂₀ FEV₁ values (ml) in response to the 4.5% NaCl challenge on the control day for the 11 asthmatic subjects are listed in the table.

Beta₂ adrenoceptor agonists were withheld for more than six hours, ipratropium bromide for more than eight hours and theophylline for more than 12 hours before the 4.5% NaCl challenge. Six of the 11 subjects had been taking beclomethasone dipropionate by inhalation on a daily basis for at least eight weeks. This was also withheld for at least six hours before the challenge. All subjects were atopic and had a positive skin reaction to one or more of eight common allergens. Subjects were asked about recent symptoms of wheeze or dyspnoea. If there was an increase in these symptoms in the previous 24 hours, testing did not proceed. The protocol was fully explained and consent was obtained in writing from each subject. The study was approved by the Ethics Committee of the Royal Prince Alfred Hospital.

METHODS

Frusemide and its vehicle

Ampoules of frusemide (Lasix; Hoechst, Germany) each containing 20 mg frusemide in 2 ml (10 mg/ml) with a pH of 9 were used in the study. The vehicle was 0.9% NaCl adjusted daily by the investigator to a pH of 8.9–9.2 by adding 0.01 mol/l sodium hydroxide. A pH meter (Radiometer PHM 62, Copenhagen, Denmark) was used to measure the pH of the frusemide and its vehicle. The osmolality of the frusemide and its vehicle was 303 mosmol as measured by freezing point depression (Advanced Cryomatic Osmometer, Model Advanced Instruments, Massachusetts, USA). Both the active and vehicle solutions were given to a pharmacist with the randomised code and one returned to the investigator to administer to the subject. This was a double blind vehicle controlled randomised crossover study.

Delivery of frusemide and its vehicle

A Fisoneb ultrasonic nebuliser (Fisons, Rochester, New York, USA) producing a

dense aerosol with a mass median aerodynamic diameter of 4.7 µm was used to deliver both the frusemide and the vehicle. The output of the nebuliser was placed on the maximum setting for the duration of the study.

To give a dose of frusemide similar to that used in published studies^{1–3 5 6 8} 6 ml of frusemide or vehicle was placed in the Fisoneb and inhaled for seven minutes. The aerosol was delivered via a mouthpiece while the subject wore a nose clip. The unit was weighed (Sartorius Analytic, Gottingen, Germany) before and after nebulisation and a stopper placed in the output hole during weighing to reduce loss of volume by evaporation. The difference in weight change was recorded as the amount of frusemide or vehicle nebulised. The mean (SD) amount of frusemide nebulised was 33.2 (3.8) mg.

AEROSOL CHALLENGE WITH 4.5% NaCl

The protocol used to perform the 4.5% NaCl challenge is described in detail by Smith and Anderson.¹⁶ The maximum dose of aerosol delivered, however, was approximately 2.5 times greater than in the original protocol and spirometric values were recorded in duplicate only one minute (not one and a half minutes) after each challenge period. The highest FEV₁ measured one minute after each challenge period was then used in the analysis of the data.

The 4.5% NaCl challenge aerosol was generated by a MistO₂gen Ultrasonic Nebuliser (Timeter, Pennsylvania, USA). Subjects inhaled the aerosols at resting ventilation rates through a two way valve (Hans Rudolf 2700, Kansas City, Missouri, USA) and mouthpiece connected to the ultrasonic nebuliser by internally non-corrugated tubing 67.5 cm in length with an internal diameter of 22 mm. This unit was weighed before and after the final dose of aerosol had been delivered.

Subjects inhaled the challenge aerosol for half a minute and waited one minute and then spirometry was performed. If there was a 20% fall in FEV₁ from the baseline FEV₁ the challenge was stopped and the subject included in the study. If a 20% fall was not recorded, the challenge continued for further intervals of one, two, four, eight, eight and eight minutes or until the provoking dose causing a 20% fall in FEV₁ (PD₂₀ FEV₁) could be recorded.

STUDY DESIGN

Subjects were required to attend the laboratory on three occasions. On the control day subjects were accepted into the study if a PD₂₀ FEV₁ in response to the 4.5% NaCl aerosol challenge was recorded. They then returned for two further visits when either frusemide or the vehicle was administered before the challenge with 4.5% NaCl.

A minimum of three days separated all visits to ensure that the medication had cleared from the airways. The maximum time between visits was eight days and the study was completed in 45 days.

Control day (visit 1)

On arrival at the laboratory, spirometry (Minato Autspirometer AS500, Osaka, Japan) was performed in triplicate and then repeated ten minutes later to establish that the airway measurement was stable. The baseline FEV₁ was taken as the best FEV₁ value recorded immediately before the challenge.

Frusemide and vehicle day (visits two and three)

The protocol for visits two and three was the same as for the control day, except that subjects inhaled the frusemide or its vehicle ten minutes before the 4.5% NaCl aerosol challenge. The challenge was stopped when either a 30% fall in FEV₁ was recorded or the last dose of aerosol was delivered.

ANALYSIS OF DATA

Baseline lung function

The percentage predicted values for FEV₁ were compared before and ten minutes after either the frusemide or its vehicle was delivered. A two way analysis of variance was used to assess if there were any differences in the percentage predicted²⁰ FEV₁ on the control day and before and ten minutes after inhaling either the frusemide or its vehicle. A level of *p* below 0.05 was considered significant. A paired *t* test was used to assess the effect of frusemide or its vehicle on baseline lung function.

Changes in airway sensitivity to 4.5% NaCl challenge: PD₂₀ FEV₁

The provoking dose of 4.5% NaCl (ml) which caused a 20% fall in FEV₁ was used as an index of airway sensitivity and changes in PD₂₀ were used to assess the effects of inhaling frusemide. A paired *t* test was used to test for differences in the PD₂₀ FEV₁ between frusemide and its vehicle and between the vehicle and the control. All PD₂₀ FEV₁ values were expressed as the geometric mean. If a 20% fall in FEV₁ was not recorded on a challenge day the maximum dose of 4.5% NaCl delivered on that challenge day was recorded as the PD₂₀ value.

Doubling dose interval

Changes in PD₂₀ FEV₁ resulting from administration of frusemide when compared with its vehicle and the vehicle when compared with the control day were expressed in doubling doses (95% confidence intervals (CI)). The doubling dose interval was used to express the results because the 4.5% NaCl aerosol was generally delivered in doubling doses.

Results

Frusemide (33.2 mg) inhaled ten minutes before challenge reduced airway sensitivity (PD₂₀) to 4.5% NaCl when compared with its vehicle (fig 1). The PD₂₀ FEV₁ increased from 1.3 ml (95% CI 0.7–2.3) after administration of the vehicle to 8.2 ml (95% CI 4.7–14.1) after frusemide (*p* < 0.001, *n* = 11). This represented an increase of 2.6 (95%

CI 2.2–3.1) doubling doses in airway sensitivity as measured by PD₂₀ FEV₁. When the results after administration of the vehicle were compared with those on the control day there was a decrease of 0.6 (95% CI 0.3–1.4) doubling doses in airway sensitivity; this was not significant (fig 1).

The mean (SD) baseline percentage predicted FEV₁ was not changed ten minutes after inhalation of either frusemide, 85.4 (15.1) *v* 87.9 (14.4) or its vehicle, 85.3 (20.0) *v* 85.2 (18.1).

The mean (SD) percentage fall in FEV₁ obtained after the highest dose of 4.5% NaCl common to the three test days had been delivered was 33.3 (12.6)% for the control, 37.6 (10.6)% for the vehicle, and 7.5 (4.9)% for frusemide. This occurred at the highest common dose of 4.5% NaCl, which was 3.8 ml (95% CI 2.4–6.0).

Individual dose-response curves for the control, vehicle, and frusemide days for the 11 subjects are given in fig 2. Only one subject (No 3) did not record a PD₂₀ in response to 4.5% NaCl after inhaling frusemide. In this subject the FEV₁ fell 13.1% after a maximum dose of 30.8 ml 4.5% NaCl had been delivered.

In five of the 11 subjects (Nos 1, 2, 3, 4 and 5) an increase in FEV₁ occurred during

Figure 1 Provoking dose of 4.5% NaCl (ml) which caused a 20% fall in FEV₁ (PD₂₀ FEV₁) on the control day, and after administration of vehicle and frusemide. Where a 20% fall in FEV₁ was not recorded the maximum dose of 4.5% NaCl delivered on the challenge day was taken as the PD₂₀. The geometric mean values and 95% confidence intervals (CI) were: 1.97 (95% CI 1.16–3.35) on the control day, 1.32 (95% CI 0.74–2.34) after administration of the vehicle, and 8.17 (95% CI 4.73–14.12) after frusemide.

Figure 2 Individual dose-response curves for the 11 subjects on the control day and after administration of vehicle and frusemide. Results are expressed as the percentage fall in FEV₁ in relation to the cumulative dose of 4.5% NaCl (ml) delivered on each test day. Control, $\square$ — $\square$; vehicle, $\circ$ — $\circ$; frusemide, $\bullet$ — $\bullet$. Note: 0.01 on the x axis represents prechallenge values.

the first five minutes of inhaling 4.5% NaCl in the presence of frusemide. Of these five subjects, Nos 1 and 2 had the lowest percentage predicted FEV₁ after frusemide inhalation before 4.5% NaCl challenge (69.2% and 68.7% respectively) and they had the greatest improvement in FEV₁ (7.5% and 9.4% increase from baseline respectively).

Discussion

This study has shown that compared with its

vehicle inhaled frusemide caused a significant decrease in airway sensitivity to challenge with a 4.5% NaCl aerosol in asthmatic subjects. Evidence that a change in airway sensitivity to 4.5% NaCl had occurred after inhalation of frusemide was shown by the 2.6 doubling dose decrease in PD₂₀ FEV₁. Frusemide also significantly reduced the percentage fall in FEV₁ at the highest delivered dose of 4.5% NaCl common to the three test days when compared with its vehicle.

These results are consistent with previous

reports of the protective effect of inhaled frusemide on other airway challenges which are thought to involve changes in airway osmolarity—for example, exercise,^{1,2} hyper-ventilation,³ and water.^{5,6}

It was interesting to note that the protective effect of frusemide was overcome with continued exposure to the 4.5% NaCl. No plateau in the airway response was observed and in 10 of the 11 subjects the final percentage fall in FEV₁ was similar to the value observed on the day the vehicle was given. This contrasts with our findings with nedocromil sodium, in which half the subjects studied had their airway responses completely inhibited or a plateau occurred in their airway response to 4.5% NaCl.²¹

A new finding in this study was the improvement in FEV₁ during the inhalation of 4.5% NaCl in the presence of frusemide. Five subjects had values for FEV₁ that were higher than baseline values. There was no change in FEV₁ in the 10 minutes after administration of frusemide and before the challenge with 4.5% NaCl. It is possible that these changes in FEV₁ early in the challenge were due to the release of a bronchodilating substance in the presence of frusemide. The effect was short but long enough to increase significantly the dose of 4.5% NaCl aerosol required to produce a 20% fall in FEV₁. Frusemide therefore reduced the sensitivity of the airways to hyperosmolar challenge but did not prevent the airways from narrowing in response to the same concentration of saline when given at a higher dose.

We think that it is unlikely that frusemide itself is acting as a functional antagonist as it provides little or no protection against histamine challenge^{2,22} and inconsistent protection against methacholine challenge^{3,23} and did not ultimately protect the airways from narrowing in this study.

There are several mechanisms by which frusemide may act to inhibit airway narrowing caused by 4.5% NaCl aerosol. One mechanism is to prevent or delay the release of inflammatory mediators from mast cells, possibly by blocking Cl⁻ site at the Na⁺/K⁺/2Cl⁻ cotransporter on the mast cell membrane.¹¹ Another mechanism is the inhibition of the Na⁺/K⁺/2Cl⁻ cotransporter²⁴ at a chloride site at the basolateral surface of the airway epithelial cell.²⁵ During challenge with 4.5% NaCl aerosol the subsequent hyperosmolarity of the airway lumen may stimulate the movement of water by osmosis first from the epithelial cells and then from the submucosa. The action of frusemide may be in reducing the potential for the submucosa to become dehydrated by blocking the movement of water by its action on the Na⁺/K⁺/2Cl⁻ cotransporter while permitting water loss from the epithelial cell in response to an osmotic stimulus. Protecting the submucosa from possible dehydration and consequent increases in osmolarity²⁶ would decrease the potential for further mediator release from mast cells and cause C afferent fibres to fire²⁷ and microvascular leakage.²⁸ All these events

could contribute to the airway narrowing provoked by the inhalation of 4.5% NaCl.

A simple explanation for the protective effect of frusemide on a wide variety of challenges may be its ability to generate prostaglandin E₂ (PGE₂),^{29,30} a relaxant of bronchial smooth muscle. PGE₂ may be released from epithelial cells secondary to hyperosmolarity, a stimulus known to generate PGE₂ production in the stomach, which is an embryologically related organ.³¹ The transient nature of the bronchodilatation is in keeping with the short half life of this substance.³² It may also be expected that, as the hyperosmolar challenge continues, mediators released from mast cells which cause bronchoconstriction override the protective effect afforded by the prostaglandins and the airways narrow. Indomethacin reduces the protective effect of inhaled frusemide against exercise induced asthma.³³ This implicates prostaglandins in the protective effect of frusemide against osmotic bronchial challenges as an increase in airway osmolarity is thought to be the mechanism causing the airways to narrow in both exercise induced asthma and asthma induced by inhaling hyperosmolar aerosols.³⁴ Other studies have shown that prostaglandin synthetase inhibitors enhance the protective effect of inhaled frusemide against metabisulphite³⁵ and ultrasonically nebulised water³⁶ bronchial challenges in asthmatic subjects. This suggests that prostaglandins may contribute to airway narrowing caused by these challenges. In our study, however, there may be a unique synergy between frusemide and hypertonic saline in releasing bronchodilator prostaglandins such as PGE₂.

This study shows that frusemide is very effective in delaying the airway narrowing induced in response to 4.5% NaCl in asthmatic subjects, thereby reducing sensitivity to hyperosmolar challenge. Although its precise mechanism of action remains uncertain, inhibition of the Na⁺/K⁺/2Cl⁻ cotransporter at the Cl⁻ site on the epithelial cells, mast cells, and nerves and enhanced release of bronchodilating substance(s), or both, may be responsible.

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