Inhaled frusemide and exercise induced asthma: evidence of a role for inhibitory prostanoids

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Abstract

Background **Inhaled frusemide protects subjects with asthma against a wide range of bronchoconstrictor challenges, including allergen, exercise and inhaled sodium metabisulphite. An investigation was designed to determine whether this protection is related to the production of inhibitory prostaglandins, such as prostaglandin E_2 (PGE_2), by studying the effect of the cyclooxygenase inhibitor indomethacin on the protection afforded by inhaled frusemide against exercise induced asthma.**

Methods **In a double blind crossover study 10 subjects with mild asthma were pretreated with indomethacin (50 mg thrice daily) or placebo capsules for three days; they then inhaled frusemide (40 mg) or placebo 10 minutes before an exercise test previously shown to cause a 20–30% fall in forced expiratory volume in one second (FEV_1).**

Results **After inhalation of placebo exercise caused a similar maximum fall in FEV_1 whether pretreatment was with placebo (26%) or indomethacin (25·2%). After inhalation of frusemide the maximum fall in FEV_1 was reduced to 14·3% after placebo pretreatment and to 21·8% after indomethacin pretreatment; the difference between placebo and indomethacin pretreatment was significant (mean difference 7·5%, 95% limits 0·6%, 14·4%). The inhibitory effect of frusemide on the response to exercise, assessed as change in FEV_1 over 30 minutes, was significantly greater with placebo (62%) than indomethacin (13%) pretreatment.**

Conclusion **These findings support a role for inhibitory prostanoids, such as PGE_2, in the beneficial effects of frusemide as a protection against exercise induced asthma.**

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Inhaled frusemide has been shown in the last few years to protect asthmatic subjects from bronchoconstriction in response to various indirectly acting stimuli, including exercise,[1] ultrasonically nebulised distilled water,[2] adenosine 5'-monophosphate,[3] sodium metabisulphite,[4] and the early and late response to allergen.[5] It has little or no effect on the bronchoconstriction following directly acting agonists, such as methacholine[3 4] and histamine.[6] The mode of action of frusemide in asthma is uncertain but is of interest as it might shed light on the pathophysiology of asthma.[7] One possibility is that frusemide inhibits epithelial sodium-potassium-chloride (Na/K/Cl) cotransport and thereby alters neural activity[4 8] or inflammatory cell activation[3 5] through a direct action or by altering the osmolarity of airway lining fluid. The lack of effect of systemic frusemide in asthma argues against this mechanism, however,[7] as does the fact that bumetanide, a more potent inhibitor of Na/K/Cl cotransport, has no effect on asthma induced by sodium metabisulphite or exercise.[9 10]

We have explored an alternative hypothesis —namely, that the effects of frusemide are due to generation of cyclooxygenase products in the airway. Frusemide enhances synthesis of prostaglandin E_2 (PGE_2),[11] and intrarenal synthesis of this prostanoid is thought to play a part in the acute vascular changes observed after intravenous administration of frusemide.[12] There is evidence that bumetanide does not share these effects when administered at equivalent diuretic doses.[13] As inhaled PGE_2 has been shown to protect against sodium metabisulphite and exercise induced asthma and the early response to allergen,[14–16] the beneficial effects of inhaled frusemide may be related to local airway production of PGE_2. We report the effect of the cyclooxygenase inhibitor indomethacin on the protection afforded by inhaled frusemide against exercise induced asthma.

Methods

SUBJECTS

We studied 10 subjects (eight male) aged 18–52 years, with mild stable atopic asthma and a 20% or more fall in FEV_1 with exercise, who required only inhaled drugs. All subjects were taking an inhaled β_2 agonist and five an inhaled corticosteroid (100–1000 μg beclomethasone a day). One subject was a current smoker and one a past smoker. All had a baseline forced expiratory volume in one second (FEV_1) of 70% predicted or more. None gave a history of aspirin induced asthma or developed bronchoconstriction in response to oral indomethacin when challenged in the laboratory. β_2 Agonists were withheld for six hours before each exercise test. Subjects gave full signed consent to participate in the study, which was approved by the City Hospital ethics committee.

Mean (95% confidence interval) values for baseline FEV_1, maximum fall in FEV_1 and change in FEV_1 over 30 minutes (AUC) for indomethacin and frusemide treatment, and within subject differences from indomethacin and frusemide for the other three treatment regimens

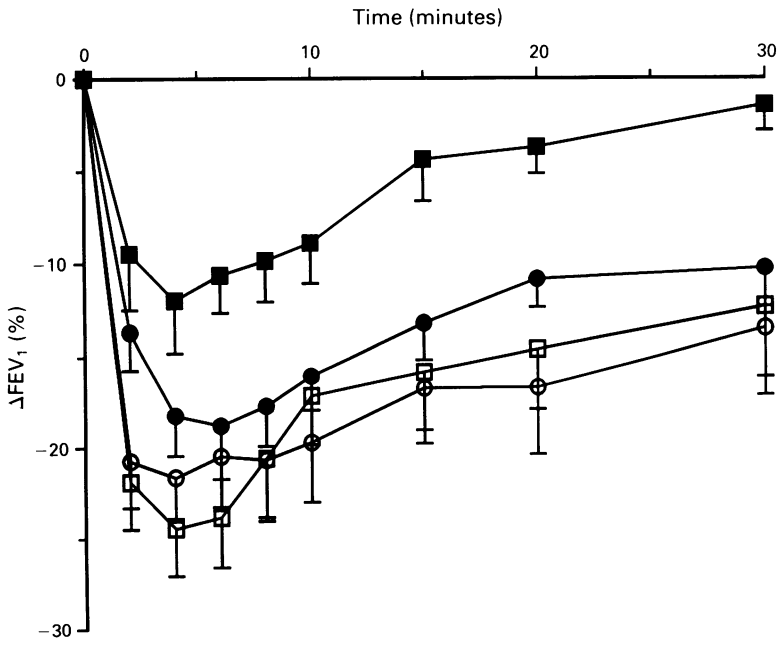
Treatment	Indomethacin + frusemide (mean values)	Mean differences from indomethacin + frusemide		
		Placebo + frusemide	Indomethacin + placebo	Placebo + placebo
Baseline FEV_1 (l)	3·26	−0·09 (0·07, −0·25)	−0·13 (0·03, −0·29)	−0·08 (0·09, −0·24)
Maximum fall in FEV_1 (%)	21·8	−7·5 (−0·6, −14·4) p < 0·05	3·5 (−3·5, 10·4)	4·3 (−2·6, 11·2)
AUC	393	−225 (−45, −405) p < 0·02	121 (−59, 301)	89 (−91, 268)

TESTS

Subjects attended for one or two assessment visits, during which practice exercise tests were performed and the work load necessary to produce a 20–30% fall in FEV_1 was established. Subjects exercised for seven minutes at room temperature on an electric treadmill (Case 12, Marquette Electronics Inc, Milwaukee) while breathing dry air through a mouthpiece connected to a Collins triple J valve, Douglas bag reservoir, and air cylinder. FEV_1 was measured on a dry bellows spirometer (Vitalograph, Buckingham) and the higher of two successive readings within 100 ml was recorded.

PROTOCOL

Subjects attended for an identical exercise test on four study days four to seven days apart at the same time of day. Before each visit the subject was asked to take indomethacin 50 mg three times daily or matched placebo for two days before and on the day of the challenge, the last dose being taken one hour before the exercise test. Ten minutes before the exercise test subjects inhaled frusemide 40 mg (made up to 5 ml with normal saline) or placebo solution (normal saline matched for pH and osmolarity) via a Medix electronic nebuliser (output 1 ml/min). Subjects inhaled through a face mask at tidal volume until the nebuliser was dry. FEV_1 measurements were taken before and five and 10 minutes after inhalation and at intervals for 30 minutes after exercise.



Percentage change in FEV_1 (ΔFEV_1) against time after exercise. Points represent mean (SEM) percentage change at each time point. Closed circle—indomethacin and inhaled frusemide; open circle—indomethacin and inhaled placebo; closed square—placebo and inhaled frusemide; open square—placebo and inhaled placebo.

ANALYSIS

The airway response to exercise was assessed as the maximum percentage fall in FEV_1 and as the area under the plot of percentage change in FEV_1 against time (AUC), the FEV_1 immediately before exercise (10 minutes after inhalation) being used as baseline. Baseline FEV_1, maximum percentage fall in FEV_1, and AUC were compared within subjects by analysis of variance by means of the generalised linear interactive modelling (GLIM) statistical package. FEV_1 values before and after inhalation were compared by a paired *t* test. The inhibitory effect of frusemide on exercise induced asthma was estimated by expressing the difference in AUC over 30 minutes between the treatment and placebo day as a percentage of the placebo AUC. The percentage inhibition was not normally distributed and is expressed as a median and range; it was compared within subjects by the Wilcoxon signed rank sum test.

Results

There was a small increase in FEV_1 after inhalation of frusemide following indomethacin pretreatment (mean 0·16 l, 95% confidence limits (CL) 0·035, 0·289; p < 0·02) but the pre-exercise (baseline) FEV_1 did not differ on any of the treatment days (table).

After inhalation of placebo exercise caused a similar mean maximum fall in FEV_1 with placebo pretreatment (26%) and indomethacin pretreatment (25·2%). After inhalation of frusemide with placebo pretreatment the mean maximum fall in FEV_1 was reduced to 14·3% (p < 0·01). When frusemide was given with indomethacin pretreatment the fall in FEV_1 was 21·8%, which did not differ from the mean maximum fall on either of the placebo inhalation days (table and figure). It did, however, differ significantly from the fall after frusemide

with placebo pretreatment (mean difference 7·5%, 95% CL 0·6, 14·4%; $p < 0·05$).

The area under the plot of change in FEV_1 against time following frusemide was compared with that following placebo inhalation and pretreatment. The median reduction in AUC after frusemide was 62% with placebo pretreatment and 13% with indomethacin pretreatment; the difference was significant ($p < 0·01$).

Discussion

This study has confirmed that inhaled frusemide protects against exercise induced asthma. The 62% reduction in the airway response to exercise after inhaled frusemide is in agreement with the findings of Bianco *et al*[1] and others.[10] We also confirmed that indomethacin alone, at a dose that abolished the rise in urinary thromboxane metabolites[17] and increase in prostaglandin concentrations in bronchoalveolar lavage fluid[18] after allergen challenge in asthmatic subjects, has no effect on the airway response to exercise.[19] Our main finding was that treatment with indomethacin for three days reduced the effect of frusemide in protecting against exercise induced asthma. This reversal of the protective effects of frusemide by a cyclooxygenase inhibitor provides strong evidence for our hypothesis that the beneficial effects of frusemide in exercise induced asthma are due to production of inhibitory prostanoids.

Both PGE_2 and prostacyclin (PGI_2) are major cyclooxygenase products of human lung[20] and in animals frusemide has been shown to enhance synthesis of PGE_2 by renal tubular epithelium[11] and PGI_2 by vascular endothelium.[21] In man PGE_2 is produced by airway epithelium[22] and PGI_2 by pulmonary vascular tissue.[20] As frusemide is only effective in asthma when inhaled, enhanced production of epithelium derived PGE_2 is the more likely mechanism.

This mechanism is consistent with the lack of effect of bumetanide in asthma. Bumetanide, like frusemide, inhibits the Na/K/Cl cotransporter but, in contrast to frusemide, does not inhibit the airway response to exercise[10] or inhaled sodium metabisulphite.[9] Intrarenal synthesis of PGE_2 in response to frusemide is thought to play a part in the vascular changes observed after intravenous frusemide.[11 12 23] Frusemide stimulates production of PGE_2 by increasing availability of arachidonic acid[23] and it may in addition inhibit the conversion of PGE_2 to $PGF_{2\alpha}$ by PGE_2-9-ketoreductase[24] and the metabolism of PGE_2 by PGE_2-15-hydroxydehydrogenase.[24 25] Bumetanide has no effect on the latter enzyme[25] and this, together with the absence of peripheral vascular changes after intravenous bumetanide,[13] suggests that frusemide has a greater effect on prostaglandin synthesis than bumetanide in relation to Na/K/Cl cotransport.

Our suggestion that frusemide is acting through release of inhibitory prostanoids assumes that these prostanoids protect predominantly against bronchoconstrictor challenges that act indirectly (through mast cell degranulation, neural pathways, or inflammatory cells) rather than those that act directly on airway smooth muscle. There is some evidence to support this view. PGE_2 does not inhibit histamine induced contractions of human airway preparations in vitro and may cause contraction rather than relaxation.[26] PGE_2 does, however, have inhibitory effects on cholinergic contractions of human airway preparations after electric field stimulation[27] and in higher concentrations on lung mast cell mediator release[28] and eosinophil activation.[29] Inhaled PGE_2 inhibits the bronchoconstrictor response to exercise, ultrasonically nebulised distilled water,[15] allergen,[16] and sodium metabisulphite in subjects with mild asthma but has little or no effect on bronchial reactivity to methacholine.[14] Studies with cyclooxygenase inhibitors support a protective role for endogenous inhibitory prostanoids, showing a reduction in the refractoriness commonly observed after recovery from bronchoconstriction induced by exercise[19] and other indirect challenges.[30 31]

The fall in FEV_1 after exercise with frusemide was greater after indomethacin than placebo despite a small increase in FEV_1 on the indomethacin treatment day after inhalation of frusemide. The mechanism of this small bronchodilator response is not clear but it may represent an effect of frusemide that is independent of cyclooxygenase products.

We considered whether the interaction between indomethacin and frusemide could be explained on grounds other than inhibition of prostaglandin synthesis. Both drugs are weak organic acids and may compete for a similar transport system across the airway epithelium. This seems unlikely, however, as frusemide is effective only when inhaled, suggesting that it acts on the luminal surface of the epithelium; and such competition does not appear to occur in the kidney, where indomethacin has no effect on renal excretion of frusemide.[11]

Of the various theories that have been advanced to explain the mode of action of frusemide, none is able to explain fully its wide range of action in asthma, the lack of protection seen with bumetanide, and the fact that frusemide appears to be effective only when inhaled. Studies suggesting that it acts on neurally mediated bronchoconstriction[8 32] do not explain the effects of frusemide on the early and late response to allergen.[5] The attraction of our hypothesis that the effects of frusemide in asthma are due to production of an inhibitory prostanoid such as PGE_2 is that it offers a plausible explanation for all of these effects.

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1 Bianco S, Vaghi A, Robuschi M, Pasargiklian M. Prevention of exercise-induced bronchoconstriction by inhaled frusemide. *Lancet* 1988;ii:252–5.

2 Robuschi M, Gambard G, Spagnotto S, Vaghi A, Bianco S. Inhaled frusemide is highly effective in preventing ultrasonically nebulised water bronchoconstriction. *Pulm Pharmacol* 1989;1:187–91.

3 Polosa R, Lau LCK, Holgate ST. Inhibition of adenosine 5'-monophosphate and methacholine-induced bronchoconstriction in asthma by inhaled frusemide. *Eur Respir J* 1990;**3**:665–72.

4 Nichol GM, Alton EWFW, Nix A, Geddes DM, Chung KF, Barnes PJ. Effect of inhaled furosemide on metabisulfite and methacholine-induced bronchoconstriction and nasal potentials in asthmatic subjects. *Am Rev Respir Dis* 1990;**142**:576–80.

5 Bianco S, Pieroni MG, Refini RM, Rottoli L, Sestini P. Protective effect of inhaled furosemide on allergen-induced early and late asthmatic reactions. *N Engl J Med* 1989;**321**:1069–73.

6 Vaghi A, Robuschi M, Berni F, Bianco S. Effect of inhaled frusemide (F) on bronchial response to histamine (H) in asthmatics. *Eur Respir J* 1988;**1**(suppl 2):406S.

7 Anonymous. Inhaled frusemide and asthma [Editorial]. *Lancet* 1990;**335**:944–6.

8 Elwood W, Lotvall JO, Barnes PJ, Chung KF. Loop diuretics inhibit cholinergic and noncholinergic nerves in guinea pig airways. *Am Rev Respir Dis* 1991;**143**:1340–4.

9 O'Connor BJ, Chung KF, Chen-Wordsdell YM, Fuller RW, Barnes PJ. Effect of inhaled furosemide and bumetanide on adenosine 5'-monophosphate and sodium metabisulfite-induced bronchoconstriction in asthmatic subjects. *Am Rev Respir Dis* 1991;**143**:1329–33.

10 Duggan CJ, Dixon CMS, Ind PW. The effect of furosemide and bumetanide in exercise-induced asthma (EIA) [abstract]. *Am Rev Respir Dis* 1990;**141**(4 part 2):A474.

11 Miyanoshita A, Terada M, Endou H. Furosemide directly stimulates prostaglandin E_2 production in the thick ascending limb of Henle's loop. *J Pharmacol Exp Ther* 1989;**251**:1155–9.

12 Johnston GD, Hiatt WR, Nies AS, Payne NA, Murphy RC, Gerber JG. Factors modifying the early nondiuretic vascular effects of frusemide in man. The possible role of renal prostaglandins. *Circ Res* 1983;**53**:630–5.

13 Johnston GD, Nicholls DP, Kondowe GB, Finch MB. Comparison of the acute vascular effects of frusemide and bumetanide. *Br J Clin Pharmacol* 1986;**21**:359–64.

14 Pavord ID, Wisniewski A, Mathur R, Wahedna I, Knox A, Tattersfield AE. Effect of inhaled prostaglandin E_2 on bronchial reactivity to sodium metabisulphite and methacholine in subjects with asthma. *Thorax* 1991;**46**:633–7.

15 Pasargiklian M, Bianco S, Allegra L, Moavero NE, Petrigni G, Robuschi M, *et al*. Aspects of bronchial reactivity to prostaglandins and aspirin in asthmatic patients. *Respiration* 1977;**34**:79–91.

16 Pasargiklian M, Bianco S, Allegra L. Clinical, functional and pathogenetic aspects of bronchial reactivity to prostaglandins $F_{2\alpha}$, E_1 and E_2. *Advances in Prostaglandin and Thromboxane Research* 1976;**1**:461–75.

17 Sladek K, Dworski R, Fitzgerald GA, Buitkus KL, Block FJ, Marney SR, *et al*. Allergen-stimulated release of thromboxane A_2 and leukotriene E_4 in humans. Effect of indomethacin. *Am Rev Respir Dis* 1990;**141**:1441–5.

18 Dworski R, FitzGerald G, Roberts L, Oates J, Schwartz L, Sheller JR. Eicosanoid formation in atopic human lung: effect of indomethacin. *Am Rev Respir Dis* 1988;**137**:375.

19 O'Byrne PM, Jones GL. The effect of indomethacin on exercise-induced bronchoconstriction and refractoriness after exercise. *Am Rev Respir Dis* 1986;**134**:69–72.

20 Holtzman MJ. Arachidonic acid metabolism. Implications of biological chemistry for lung function and disease. *Am Rev Respir Dis* 1991;**143**:188–203.

21 Lundergan CF, Fitzpatrick TM, Rose JC, Ramwell RN, Kot PA. Effect of cyclooxygenase inhibition on the pulmonary vasodilator response to furosemide. *J Pharmacol Exp Ther* 1988;**246**:102–6.

22 Churchill L, Chilton FH, Resau JH, Bascom R, Hubbard WC, Proud D. Cyclooxygenase metabolism of endogenous arachidonic acid by cultured human tracheal epithelial cells. *Am Rev Respir Dis* 1989;**140**:449–54.

23 Weber PC, Scherer B, Larsson C. Increase of free arachidonic acid by frusemide in man as the cause of prostaglandin and renin release. *Eur J Pharmacol* 1977;**41**: 329–32.

24 Stone KJ, Hart M. Inhibition of renal PGE_2-9-ketoreductase by diuretics. *Prostaglandins* 1976;**12**:197–207.

25 Oliw E. Prostaglandins and kidney function. *Acta Physiol Scand* 1979;**461**(suppl):1–55.

26 Haye-Legrand I, Cerrina J, Raffestin B, Labat C, Boullet C, Bayol A, *et al*. Histamine contractions of isolated human airway smooth muscle preparations: role of prostaglandins. *J Pharmacol Exp Ther* 1986;**239**:536–41.

27 Ito I, Suzuki H, Aizawa H, Hirose T, Hakoda H. Prejunctional inhibitory action of prostaglandin E_2 on excitatory neuro-effector transmission in the human bronchus. *Prostaglandins* 1990;**39**:639–55.

28 Peters SP, Schulman ES, MacGlashan DW, Schleimer RP, Newball HH, Lichtenstein LM. Pharmacological and biochemical studies of human lung mast cells. *J Allergy Clin Immunol* 1982;**69**:150.

29 Giembycz MA, Kroegel C, Barnes PJ. Prostaglandin E_2 inhibits platelet activating factor-induced eosinophil activation [abstract]. *Am Rev Respir Dis* 1990;**141**(4 part 2):A396.

30 Mattoli S, Foresi A, Corbo GM, Valente S, Ciappi G. The effect of indomethacin on the refractory period occurring after the inhalation of ultrasonically nebulized distilled water. *J Allergy Clin Immunol* 1987;**79**:678–83.

31 Dorsch W, Frey L. Allergen tachyphylaxis of guinea pigs in vivo: a prostaglandin E mediated phenomenon? *Naunyn-Schmiedeberg's Arch Pharmacol* 1981;**317**:351–6.

32 Ventresca PG, Nichol GM, Barnes PJ, Chung KF. Inhaled furosemide inhibits cough induced by low chloride content solutions but not by capsaicin. *Am Rev Respir Dis* 1990;**142**:143–6.