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 E2 (PGE2), 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 (FEV1). Results After inhalation of placebo exercise caused a similar maximum fall in FEV1, whether pretreatment was with placebo (26%) or indomethacin (25-2%). After inhalation of frusemide the maximum fall in FEV1 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 FEV1 over 30 minutes, was significantly greater with placebo (62%) than indomethacin (13%) pretreatment. Conclusion These findings support a role for inhibitory prostanoids, such as PGE2, in the beneficial effects of frusemide as a protection against exercise induced asthma.

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 methacholine6 and histamine.7 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 activity8 or inflammatory cell activation9 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 E2 (PGE2),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 PGE2 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 PGE2. 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 FEV1, with exercise, who required only inhaled drugs. All subjects were taking an inhaled β2 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 (FEV1) 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. β2 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<sub>1</sub>, maximum fall in FEV<sub>1</sub>, and change in FEV<sub>1</sub>, over 30 minutes (AUC) for indomethacin and frusemide treatment, and within subject differences from indomethacin and frusemide for the other three treatment regimens.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Indomethacin + frusemide (mean values)</th>
<th>Mean differences from indomethacin + frusemide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline FEV&lt;sub&gt;1&lt;/sub&gt; (L)</td>
<td>3.26</td>
<td>Placebo + frusemide</td>
</tr>
<tr>
<td>Maximum fall in FEV&lt;sub&gt;1&lt;/sub&gt; (%)</td>
<td>21.8</td>
<td>-0.09 (0.07, -0.25)</td>
</tr>
<tr>
<td>AUC</td>
<td>393</td>
<td>3.5 (-0.6, -14.4)</td>
</tr>
</tbody>
</table>

**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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> measurements were taken before and five and 10 minutes after inhalation and at intervals for 30 minutes after exercise.

**Analysis**

The airway response to exercise was assessed as the maximum percentage fall in FEV<sub>1</sub>, and as the area under the plot of percentage change in FEV<sub>1</sub> against time (AUC), the FEV<sub>1</sub> immediately before exercise (10 minutes after inhalation) being used as baseline. Baseline FEV<sub>1</sub>, maximum percentage fall in FEV<sub>1</sub>, and AUC were compared within subjects by analysis of variance by means of the generalised linear interactive modelling (GLIM) statistical package. FEV<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> did not differ on any of the treatment days (table).

After inhalation of placebo exercise caused a similar mean maximum fall in FEV<sub>1</sub>, with placebo pretreatment (26%) and indomethacin pretreatment (25.2%). After inhalation of frusemide with placebo pretreatment the mean maximum fall in FEV<sub>1</sub> was reduced to 14.3% (p < 0.01). When frusemide was given with indomethacin pretreatment the fall in FEV<sub>1</sub> 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
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with placebo pretreatment (mean difference 7.5%, 95% CL 0.6, 14.4%, p < 0.05).

The area under the plot of change in FEV1 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 al20 and others.21 We also confirmed that indomethacin alone, at a dose that abolished the rise in urinary thromboxane metabolites22 and increase in prostaglandin concentrations in bronchoalveolar lavage fluid23 after allergen challenge in asthmatic subjects, has no effect on the airway response to exercise.24 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 PGE2 and prostacyclin (PGI2) are major cyclooxygenase products of human lung22 and in animals frusemide has been shown to enhance synthesis of PGE2 by renal tubular epithelium25 and PGI2 by vascular endothelium.26 In man PGE2 is produced by airway epithelium27 and PGI2 by pulmonary vascular tissue.28 As frusemide is only effective in asthma when inhaled, enhanced production of epithelium derived PGE2 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 exercise29 or inhaled sodium metabolite.30 Intrarenal synthesis of PGE2 in response to frusemide is thought to play a part in the vascular changes observed after intravenous frusemide.31 32 Frusemide stimulates prostaglandin synthesis,33 34 frusemide by increasing availability of arachidonic acid and it may in addition inhibit the conversion of PGE2 to PGF2α, by PGE2-9-ketoreductase35 and the metabolism of PGE2 by PGE2-15-hydroxydehydrogenase.36 37 Bumetanide has no effect on the latter enzyme38 and this, together with the absence of peripheral vascular changes after intravenous bumetanide,39 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. PGE2 does not inhibit histamine induced contractions of human airway preparations in vitro and may cause contraction rather than relaxation.26 PGE does, however, have inhibitory effects on cholinergic contractions of human airway preparations after electric field stimulation27 and in higher concentrations on lung mast cell mediator release30 and eosinophil activation.29 Inhaled PGE2 inhibits the bronchoconstrictor response to exercise, ultrasonically nebulised distilled water,19 allergen,40 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 exercise19 and other indirect challenges.20 31

The fall in FEV1 after exercise with frusemide was greater after indomethacin than placebo despite a small increase in FEV1, 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.41

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 bronchoconstriction42 do not explain the effects of frusemide on the early and late reduction of PGE2. The attraction of our hypothesis that the effects of frusemide in asthma are due to production of an inhibitory prostanooid such as PGE2 is that it offers a plausible explanation for all of these effects.

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