Effect of inhaled frusemide on responses of airways to bradykinin and adenosine 5'-monophosphate in asthma

K Rajakulasingam, R Polosa, M K Church, P H Howarth, S T Holgate

Abstract

Background – Inhaled frusemide exerts a protective effect against bronchoconstriction induced by several indirect stimuli in asthma. This effect could be caused by interference with neural pathways. The effect of inhaled frusemide on bronchoconstriction induced by inhaled bradykinin, which is thought to cause bronchoconstriction via neural mechanisms, was studied and compared with the effects of adenosine 5'-monophosphate (AMP) which probably produces its airway effects by augmenting mast cell mediator release and interfering with neural pathways.

Methods – Patients first underwent AMP and bradykinin challenges. They were then studied in a randomised, placebo controlled, double blind fashion. Ten atopic asthmatic subjects, studied on four days, were pretreated with inhaled frusemide (40 mg) or placebo for 10 minutes, five minutes before challenge with increasing concentrations of nebulised AMP or bradykinin.

Results – On the open visit days the provocative concentrations required to reduce forced expiratory volume in one second (FEV₁) by 20% from baseline (PC20) for AMP and bradykinin were 16-23 (1-42–67-16) and 2-75 (0-81–6-6) mg/ml. There was a significant correlation between baseline AMP and bradykinin PC20 values. For AMP the geometric mean PC20 values following pretreatment with inhaled frusemide and matched placebo were 80-97 (9-97–>400-0) and 14-86 (2-6–104-6) mg/ml respectively (95% CI 0-49 to 0-98). For bradykinin the geometric mean PC20 values following pretreatment with inhaled frusemide and matched placebo were 13-22 (2-53–>16-0) and 2-52 (0-45–5-61) mg/ml respectively (95% CI 0-43 to 1-01). Frusemide afforded 5-45 and 5-24 fold protection against AMP and bradykinin-induced bronchoconstriction respectively. Furthermore, there was a significant correlation between protection afforded to the airways against AMP and bradykinin.

Conclusions – These data suggest that inhaled frusemide affords protection against bradykinin-induced bronchoconstriction which is comparable to that against AMP, supporting a common mechanism of action for frusemide.
The effect of inhaled frusemide on bradykinin-induced bronchoconstriction has not been reported. In this study we have investigated the effect of inhaled frusemide against bronchoconstriction provoked by bradykinin and compared it with that of AMP which probably produces its airway effects by augmenting mast cell mediator release and excitation of neural pathways. We have also assessed the degree of protection afforded by frusemide against both stimuli as, if a similar degree of protection was found, this would support a common mechanism of action for this drug in the airways.

Methods

SUBJECTS

Ten asthmatic subjects (eight males) with a mean (SE) age 25·5 (2·4) years participated in the study. All subjects were non-smokers with atopic asthma as judged by at least one weal >3 mm on skin prick testing with *Derma-tophagoides pteronyssinus*, house dust, mixed grass pollen, cat fur, and feathers (Bencard, Brentford, Middlesex, UK). Their baseline FEV₁ was >75% of predicted values and none were receiving oral corticosteroids or theophylline, but five were on low dose inhaled beclometasone (table 1). Treatment with inhaled β₂ agonists was withdrawn at least eight hours before each visit to the laboratory, although subjects were allowed to continue inhaled corticosteroids as usual. Patients were not studied within four weeks of an upper respiratory tract infection or exacerbation of their asthma and all visits to the laboratory were carried out at the same time of day. The study was approved by the Southampton University and Hospitals ethical subcommittee and written informed consent was given by all subjects involved in the study.

BRONCHIAL PROVOCATION TESTS

**Bronchial challenge with adenosine**

5'-monophosphate and bradykinin

Adenosine 5'-monophosphate (AMP) (Sigma Chemical Co., St Louis, USA) was made up in 0·9% sodium chloride in a series of doubling concentrations ranging from 0·39 to 400 mg/ml (4·48–115·1 mmol/l). Bradykinin triacetate acid (Nova Biochem, Nottingham, UK) was dissolved in phosphate buffered saline (pH 7·4) to produce a concentration range of 0·03–16 mg/ml (0·028–15·09 mmol/l). To avoid loss of bradykinin through oxidation and adherence to plastic surfaces the stock solution was stored at 4°C before use and broncho-provocation was performed within 30 minutes of preparing the dilutions. The solutions were administered as aerosols generated from a starting volume of 3 ml in a Cirrus Mininebuliser (Intersurgical, Middlesex, UK) driven by compressed air at 8 l/min. Under these conditions the nebuliser generates an aerosol with a mass median particle diameter of 4·1 μm.26

Bronchial challenge was performed with a technique modified from that of Chai et al.23 Measurements of FEV₁ were made using a dry wedge spirometer (Vitalograph, Buckinghamshire, UK). Before challenge, after 15 minutes rest, three baseline measurements of FEV₁ were made at three minute intervals and the highest value recorded. Subjects then inhaled nebulised 0·9% sodium chloride (saline) taking five slow breaths from functional residual capacity (FRC) to full inspiration, and FEV₁ measurements performed at one and three minutes, the higher value being recorded. If this value was 10% of the initial FEV₁, then bronchial provocation with AMP or bradykinin was undertaken. Increasing concentrations of agonists were inhaled at five minute intervals until the FEV₁ had fallen by >20% of the post-diluent baseline value, or until the highest concentrations of agonist had been administered. The percentage fall in FEV₁, from post-diluent baseline was plotted against the cumulative concentration of agonist administered, and the provocative concentration of agonist required to produce a 20% fall in FEV₁ from the post-diluent baseline (PC₂₀ FEV₁) derived by linear interpolation of the last two points.

**Table 1 Characteristics of subjects**

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Age (years)</th>
<th>PC₂₀ histamine (mg/ml)</th>
<th>FEV₁ (%) predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>0·23</td>
<td>96·0</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>0·5</td>
<td>105·0</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>0·5</td>
<td>86·0</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>1·0</td>
<td>95·0</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>0·25</td>
<td>98·0</td>
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<td>6</td>
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<td>1·3</td>
<td>78·0</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>2·4</td>
<td>82·0</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>0·44</td>
<td>94·0</td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>0·27</td>
<td>105·0</td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td>1·2</td>
<td>102·0</td>
</tr>
<tr>
<td>Mean (SE)</td>
<td>25·5 (2·4)</td>
<td>0·60</td>
<td>94·1 (3·1)</td>
</tr>
</tbody>
</table>

* Geometric mean.
Effect of inhaled frusemide on bradykinin-induced bronchoconstriction

Table 2 PC\textsubscript{20} values (mg/ml) for AMP and bradykinin (BK)

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Baseline PC\textsubscript{20}AMP</th>
<th>Post-placebo PC\textsubscript{20}AMP</th>
<th>Post-frusemide PC\textsubscript{20}AMP</th>
<th>Baseline PC\textsubscript{20}BK</th>
<th>Post-placebo PC\textsubscript{20}BK</th>
<th>Post-frusemide PC\textsubscript{20}BK</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>43-72</td>
<td>80-5</td>
<td>&gt;400-0</td>
<td>4-4</td>
<td>2-6</td>
<td>6-8</td>
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<tr>
<td>2</td>
<td>12-8</td>
<td>2-66</td>
<td>20-69</td>
<td>0-81</td>
<td>0-45</td>
<td>2-25</td>
</tr>
<tr>
<td>3</td>
<td>22-38</td>
<td>7-11</td>
<td>85-1</td>
<td>6-6</td>
<td>3-7</td>
<td>11-98</td>
</tr>
<tr>
<td>4</td>
<td>11-92</td>
<td>6-11</td>
<td>9-97</td>
<td>5-85</td>
<td>3-38</td>
<td>&gt;16-0</td>
</tr>
<tr>
<td>5</td>
<td>18-13</td>
<td>22-25</td>
<td>79-31</td>
<td>1-9</td>
<td>2-64</td>
<td>7-7</td>
</tr>
<tr>
<td>6</td>
<td>17-88</td>
<td>15-55</td>
<td>122-38</td>
<td>5-38</td>
<td>4-4</td>
<td>&gt;16-0</td>
</tr>
<tr>
<td>7</td>
<td>13-04</td>
<td>12-02</td>
<td>153-26</td>
<td>1-47</td>
<td>0-63</td>
<td>&gt;16-0</td>
</tr>
<tr>
<td>8</td>
<td>20-84</td>
<td>49-75</td>
<td>59-2</td>
<td>2-01</td>
<td>5-61</td>
<td>7-42</td>
</tr>
<tr>
<td>9</td>
<td>1-42</td>
<td>0-26</td>
<td>1-12</td>
<td>1-08</td>
<td>3-63</td>
<td>&gt;13-31</td>
</tr>
<tr>
<td>10</td>
<td>67-16</td>
<td>104-6</td>
<td>&gt;400-0</td>
<td>5-41</td>
<td>4-78</td>
<td>&gt;16-0</td>
</tr>
<tr>
<td>Geometric mean (range)</td>
<td>16-23 (1-42-67)</td>
<td>2-52 (2-6-104-6)</td>
<td>9-97-&gt;400</td>
<td>0-81-66</td>
<td>0-45-561</td>
<td>2-53-&gt;16-0</td>
</tr>
</tbody>
</table>

Methods of dose administration

The dose of frusemide delivered to the mouth was calculated by differential weighing and on four of the occasions amounted to 2800 (2.6) mg/ml. Five minutes after inhaling the frusemide or placebo a concentration response study was performed with one of the two agonists as described before.

DATA ANALYSIS

For FEV\textsubscript{1}, the lowest of the values recorded with each inhaled concentration of agonist was used for analysis. Baseline FEV\textsubscript{1}, measurements before bronchial challenges were compared between study days by two way analysis of variance (ANOVA). FEV\textsubscript{1}, values prior to bronchial challenges before and after treatment were compared within each study day using the Student’s t test for paired data. The airways response to AMP and bradykinin at each agonist concentration was expressed as the percentage change in FEV\textsubscript{1}, from the post-dilute baseline value. Values of PC\textsubscript{20}, bradykinin and AMP were logarthmically transformed and compared using the Student’s t test for paired data and the results expressed as 95% confidence interval (CI). If a 20% fall in FEV\textsubscript{1}, was not achieved by the maximum dose of agonist, the PC\textsubscript{20} was estimated as the next doubling dose.

Concentration ratios for the protective effect of frusemide against bronchoprovocation with each agonist were calculated by dividing the PC\textsubscript{20} value obtained after administration of the active drug by that obtained after placebo. The relative potency of frusemide in protecting against bronchoconstriction induced by the two agonists was analysed by comparing the concentration ratios using the Wilcoxon’s signed rank test. The relation between PC\textsubscript{20} AMP and PC\textsubscript{20} bradykinin values, and the respective PC\textsubscript{20} concentration ratios after frusemide pretreatment, were investigated by least squares linear regression analysis and Spearman’s rank correlation analysis respectively. A p value of <0.05 was accepted as the minimum level of statistical significance.

**Results**

Baseline values of FEV\textsubscript{1}, did not differ significantly between any of the six study days, the mean values being 3-68 (0-2), 3-63 (0-2), 3-58 (0-19), 3-5 (0-2), 3-6 (0-18), and 3-61 (0-24) l for open AMP and bradykinin days, AMP-active frusemide and AMP-placebo frusemide days, and bradykinin-active frusemide and bradykinin-placebo frusemide treatment days, respectively. There were no significant differences in baseline values of FEV\textsubscript{1}, after placebo or frusemide between any of the study days.

All 10 subjects exhibited bronchial responsiveness to inhaled AMP and bradykinin, the geometric mean (range) concentrations required to produce a 20% decrease in FEV\textsubscript{1}, (PC\textsubscript{20} FEV\textsubscript{1}) values being 16-23 (1-42-67) and 2-75 (0-81-66) mg/ml, respectively. On a molar basis AMP was therefore approximately 17.9 (4-0-48-3) fold less potent than bradykinin in reducing FEV\textsubscript{1}. A significant correlation was observed between baseline PC\textsubscript{20} AMP and bradykinin values (r = 0.58, p < 0.05). However, there was no correlation between bronchial responsiveness to histamine and either AMP or bradykinin.

Inhaled frusemide had a significant protective effect against the fall in FEV\textsubscript{1}, produced by AMP, the geometric mean (range) PC\textsubscript{20} AMP increasing from 14-86 (2-6-104-6) after placebo to 80-97 (9-97->400-0) mg/ml after frusemide (95% CI 0-49 to 0-98) (fig 1, table 2). The same dose of frusemide was also effective in protecting against bradykinin-induced bronchoconstriction, the geometric mean (range) PC\textsubscript{20} increasing from 2-52 (0-45-5-61) mg/ml after placebo to 13-22 (2-53->16-0) mg/ml following frusemide pretreatment (95% CI 0-43 to 1-01) (fig 2, table 2). When expressed as concentration ratios frusemide afforded 5-45 fold (>2.5 doubling dilution) and 5-24 fold (>2.5 doubling dilution) protection of the airways against AMP and bradykinin respectively. A significant correlation existed for the capacity of frusemide to protect against bronchoconstriction provoked by AMP and by bradykinin (p = 0.05, r\textsubscript{s} = 0.51).

**Discussion**

This study confirms previous findings that inhaled bradykinin and AMP both caused dose-related bronchoconstriction in asthmatic subjects. Within the group a significant correlation existed between the baseline PC\textsubscript{20} values for AMP and bradykinin. We have also shown that frusemide administered in an inhaled dose of about 28 mg (of which about 10% reaches the airways) produced an approx-
imately fivefold protection against bronchoconstriction induced by both AMP and bradykinin. Since these agonists are considered to produce bronchoconstriction by differing mechanisms — bradykinin involving sensory nerve stimulation and tachykinin release, and AMP augmenting mast cell mediator release and, to a lesser extent, via neural stimulation — the mechanism of protection afforded by frusemide is puzzling.

The lack of correlation between histamine and either bradykinin or AMP bronchial reactivities supports indirect mechanism(s) of action for bradykinin and AMP. Three subjects (nos 2, 3, and 8) have shown more than a doubling concentration difference in PC20 for AMP on baseline and placebo days (table 2). We have previously shown, however, that coefficients of repeatability were within single doubling concentrations for AMP and bradykinin.6,19

Since the original description by Bianco et al of the inhibitory effect of inhaled but not oral frusemide against exercise-induced asthma,3
Effect of inhaled frusemide on bradykinin-induced bronchoconstriction

Figure 2 Bronchoconstrictor response to bradykinin in all 10 subjects following pretreatment with frusemide (○) and placebo (●).

Further studies have shown that it affords little or no protection against histamine-induced bronchoconstriction and only minor protection against methacholine. In providing protection against different “indirect” stimuli such as allergen, fog, exercise, cold air, sodium metabisulphite, AMP, and now bradykinin, however, the inhibitory effect must embrace a mechanism or mechanisms common to all these stimuli.

Frusemide is known to produce some of its effect in the kidney by the secondary production of endogenous prostanoids and in bovine tracheal mucosa it produces PGE₂. Furthermore, a recent study has shown that indomethacin reduced the protective effect of frusemide against exercise-induced asthma, suggesting release of prostaglandins such as PGE₂ and PGI₂ which are both potent functional antagonists. Both human airways and pulmonary vascular endothelial cells are rich sources of PGI₂ and PGE₂. In asthma, inhaled PGI₂ has been shown to afford short term protection against stimuli such as exer-
cise, fog, and PGD, in the absence of any consistent bronchodilator effect. Similarly, inhaled PGE, protects against the contractile effect of sodium metabolisulphite and methacholine in asthma. Another loop diuretic, bumetanide, which is also known to induce the generation of prostanooids and act via the Na-K-Cl cotransporter mechanism in renal tubules, has not been shown to afford protection against AMP. This indicates that the airway effects of frusemide may be mediated through pathways independent of the shared properties with bumetanide. However, the lack of effect of bumetanide might also have been due to its pharmacokinetic properties.

The bronchoconstrictor actions of AMP and bradykinin are inhibited by disodium cromoglycate and nedocromil sodium and attenuated by anticholinergic agents, suggesting that excitation of neural pathways may underlie these responses. Furthermore, FK 224, a neurokinin 1 antagonist, has been shown to afford protection against bradykinin-induced bronchoconstriction in asthma. Mast cell release of histamine plays no significant part in bradykinin-induced bronchoconstriction in asthma. While antihistamines have been shown to afford protection against AMP-induced bronchoconstriction, this protection was incomplete and additional pathways are likely to be involved. One possibility is that vagal reflexes may contribute directly to the bronchoconstriction induced by inhaled purines in asthma, as suggested by Pauwels et al. Furthermore, Polosa et al have shown that inhaled ipratropium bromide affords a significant 2.5 fold protection against AMP-induced bronchoconstriction in asthma. However, the exact stimulus prompting the vagal reflex activation remains to be clarified. Possibilities include a direct effect of purines in the stimulation of cholinergic reflexes, or indirect activation through the release of mast cell mediators such as histamine and prostanoids. In support of the ability of histamine to influence vagal airway tone, both atropine and inhaled hexamethonium bromide have been shown to be effective inhibitors of histamine-induced bronchoconstriction through cholinergic and ganglionic blockade respectively.

Peachell et al have shown that, in immunologically activated human lung mast cells, adenosine not only enhances histamine release but also potentiates production of prostanooids. Furthermore, in asthma, cyclo-oxygenase blockade has been shown to inhibit the bronchoconstrictor response provoked by inhaled purines. As the effects of prostanooids on airway calibre are known to be mediated in part by vagal reflexes, it is possible that adenosine-induced production of prostanooids from mast cells contributes to bronchoconstriction via cholinergic pathways. Some evidence has accumulated to indicate that inhaled frusemide might alter neural activity in the airways. In healthy subjects inhaled frusemide inhibits cough responses induced by low chloride aerosols. Further evidence derives from the observation that both frusemide and bumetanide inhibit the airway smooth muscle contraction induced by stimulation of non-cholinergic non-adrenergic nerves independent of cyclo-oxygenase production.

Our observations of a similar degree of protection produced by frusemide against the airways response to inhaled AMP and bradykinin, and the finding of a significant correlation between the degree of protection against both stimuli, suggest that inhaled frusemide may act in a manner analogous to frusemide. Since many of the other "indirect" stimuli also involve neural reflexes to a greater or lesser extent, this seems the most likely component of the bronchoconstrictor response that is frusemide sensitive.

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5. 5 Polosa R, Lau LCK, Holgate ST. Inhibition of adenosine 5′-monophosphate (AMP) and methacholine-induced bronchoconstriction in asthma by inhaled frusemide. Eur Respir J 1990;5:665-72.
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