Effect of acetazolamide and amiloride against sodium metabisulphite-induced bronchoconstriction in mild asthma

B J O'Connor, C T Yeo, Y M Chen-Worsdell, P J Barnes, K F Chung

Abstract

Background — Inhaled frusemide but not bumetanide, another loop diuretic, reduces bronchial responsiveness to sodium metabisulphite (MBS). To investigate whether the effect of frusemide could be mediated through mechanisms other than Na⁺/K⁺/Cl⁻ cotransporter inhibition, the effects of amiloride — an inhibitor of sodium channels in the airway epithelium — and of acetazolamide — a specific inhibitor of carbonic anhydrase — against MBS challenge were studied.

Methods — In two separate randomised double blind placebo controlled studies, 10 subjects with mild asthma attended on four separate occasions to inhale 7.5 mg amiloride or matched placebo, and 500 mg acetazolamide or placebo, immediately before MBS challenge. The concentration of MBS required to cause a 20% fall in baseline FEV₁ (PC₂₀) was measured.

Results — Amiloride and acetazolamide had no effect on baseline FEV₁. Amiloride had no effect against MBS challenge, but acetazolamide increased —log PC₂₀ from a mean (SE) of 0.75 (0.09) to 0.98 (0.06) representing a 0.77 (0.24) doubling dose increase.

Conclusions — These results suggest that carbonic anhydrase activity in the airways, but not sodium flux, modulates bronchial responsiveness to MBS challenge. The action of frusemide is not likely to involve inhibition of carbonic anhydrase activity.

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Administration of diuretics such as frusemide and chlorothiazide has previously been shown to improve lung function in patients with bronchopulmonary dysplasia.12 More recent studies have shown that frusemide can attenuate bronchoconstrictor responses induced by a wide range of indirect challenges including exercise, allergen, sodium metabisulphite (MBS), and adenosine in patients with asthma. Thus, diuretics may have direct effects on the airways in addition to improving cardiopulmonary function through their diuretic effects. The mechanism of action underlying the protective effects of the loop diuretic, frusemide, in the airways is unclear. Loop diuretics such as frusemide and bumetanide are potent inhibitors of the Na⁺/K⁺/2Cl⁻ cotransporter system in the ascending loop of Henle in the renal tubule.4 Because frusemide — but not the more potent loop diuretic bumetanide — inhibits indirect challenges such as MBS and adenosine,5 it seems unlikely that inhibition of the Na⁺/K⁺/2Cl⁻ cotransporter is the underlying mechanism of action of frusemide. Because frusemide has inhibitory effects on carbonic anhydrase activity,6 we hypothesised that carbonic anhydrase inhibition with acetazolamide may inhibit the bronchoconstrictor effect of MBS. We also investigated whether modification of sodium fluxes across the apical surface of the airway epithelium could be involved. Amiloride is another diuretic which inhibits the diffusion of sodium ions through sodium channels at the apical surface of airway epithelial cells.7 We therefore studied whether inhaled acetazolamide and amiloride could attenuate the bronchoconstrictor effects of inhaled MBS in asthmatic subjects.

Methods

SUBJECTS
Ten non-smoking subjects with mild asthma (five men) of mean (SE) age 31·3 (3·7) years, 93·1 (4·2)% predicted FEV₁ (table 1) gave informed consent to participate in this study which was approved by the Royal Brompton Hospital ethics committee. All patients were taking short-acting inhaled β₂ adrenergic agonists intermittently for the relief of wheeze, but none were on inhaled steroid therapy. None were smokers. All patients were stable for at least six months before entry to study.

PROTOCOL
Two separate randomised double blind placebo controlled studies were performed. Each subject participated in both studies. In the first study subjects inhaled either amiloride (7·5 mg) or matched placebo, followed five minutes later by airway challenge with MBS. The study visits were separated by at least seven days. In the second study subjects inhaled either the carbonic anhydrase inhibitor acetazolamide (500 mg) or matched placebo, followed five minutes later by MBS challenge. The study visits were also separated by at least seven days. MBS challenges were performed at the same time of the day.

Delivery of acetazolamide and amiloride
Acetazolamide solution (Diamox parenteral, Lederle Laboratories, Gosport, Hants, UK;
Acetazolamide, amiloride and MBS challenge

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Atopy</th>
<th>FEV₁ (% predicted)</th>
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<td>38</td>
<td>F</td>
<td>+</td>
<td>81-1</td>
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<tr>
<td>2</td>
<td>51</td>
<td>M</td>
<td>+</td>
<td>83-1</td>
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<td>26</td>
<td>F</td>
<td>+</td>
<td>82-5</td>
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<td>4</td>
<td>21</td>
<td>M</td>
<td>+</td>
<td>102-4</td>
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<td>30</td>
<td>M</td>
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<td>93-1</td>
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<td>10</td>
<td>21</td>
<td>M</td>
<td>+</td>
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</tr>
</tbody>
</table>

Results

In both studies inhalation of the diluents alone or of acetazolamide and amiloride caused no significant changes in FEV₁ (table 2). Mean \(-\log PC_{20}\) after amiloride (0.78 (0.08)) was not significantly altered compared with that after placebo (0.83 (0.07)). However, mean \(-\log PC_{20}\) increased significantly from 0.75 (0.09) after placebo to 0.98 (0.06) after acetazolamide (figure; p<0.02), representing a 0.77 (0.24) doubling dilution increase.

Discussion

The aim of the study was to determine whether the mechanism of action of frusemide, which has been shown to inhibit bronchoconstrictor responses to indirect challenges, may be related to an effect on sodium transport (through a sodium channel inhibitor) or on carbonic anhydrase activity. Our results suggest that this is unlikely to be occurring through significant alterations in Na⁺ entry channels at the apical surface of airway epithelial cells because amiloride was not effective in inhibiting MBS-induced bronchoconstriction. By contrast, acetazolamide caused a significant inhibition suggesting that carbonic anhydrase activity may modulate MBS-induced bronchoconstriction.

The dose of amiloride administered was in the same range as that given by App and colleagues who found a significant improvement in mucociliary clearance which occurred within the first 10 minutes and was maintained for a further 30 minutes after inhalation in patients with cystic fibrosis.⁹ The concentration of am-
iloride used would be within that described as possessing inhibitory actions against Na⁺ absorption, although the degree of dilution that occurs at the site of action is difficult to determine. Our data suggest that changes in Na⁺ transport and in airway hydration are not important mechanisms by which frusemide inhibits bronchoconstrictor challenges and are similar to those recently reported by Baldwin and colleagues. Our results are also in agreement with those of Rodwell et al who showed that inhaled amiloride did not protect against dry air challenge in asthmatic subjects in whom protection is afforded by frusemide. However, the negative results obtained with amiloride must be interpreted in the light of the fact that it was not possible to test higher concentrations of amiloride because of its relative insolubility.

Our studies with acetazolamide demonstrate that carbonic anhydrase inhibition provides some protective effect against MBS challenge. The dose of acetazolamide used afforded approximately half the protective effect observed with 40 mg of inhaled frusemide. We used the maximum concentration of acetazolamide that could be maintained in solution and acetazolamide is a very potent inhibitor of carbonic anhydrase activity in vitro. Frusemide has previously been shown to possess weak carbonic anhydrase inhibitory activity in the renal tubules as judged by its effect on bicarbonate excretion. However, because frusemide is generally more effective in inhibiting MBS-induced bronchoconstriction than acetazolamide, it is unlikely that the inhibitory effect of frusemide can be ascribed to its relatively weaker effect as an inhibitor of carbonic anhydrase. The inhibitory effect of acetazolamide is not only restricted to MBS challenges because a small but significant protective effect against bronchoconstriction induced by cold dry air challenge has recently been reported.

The mechanism by which inhibition of carbonic anhydrase activity may be inhibiting MBS-induced bronchoconstriction in asthmatic patients is not clear. We have recently shown that acetazolamide can inhibit non-adrenergic non-cholinergic contractile responses induced by electrical stimulation in guinea pig bronchial strips by preventing the release of contractile neuropeptides from sensory nerve endings. Acetazolamide is known to inhibit carbonic anhydrase activity in afferent peripheral nerves of rodents. Although the precise mechanism of MBS-induced bronchoconstriction is not clear, it is possible that a non-cholinergic non-adrenergic neural pathway may be involved and acetazolamide may inhibit these pathways.

16 Cammer W, Tansey FA. Immunocytochemical localization of carbonic anhydrase in myelinated fibres in peripheral nerves of rat and mouse. J Histochem Cytochem 1987;35: 865-70.
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