Eur Respir J
1992, 5, 1189–1192

The effect of amiloride on the airway response to metabisulphite in asthma: a negative report

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The effect of amiloride on the airway response to metabisulphite in asthma: a negative report. D.R. Baldwin, K.L. Grange, I. Pavord, A.J. Knox.

ABSTRACT: Frusemide, a loop diuretic, has been shown to potently inhibit several indirect bronchoconstrictor challenges in asthma. The mechanism by which nebulized frusemide protects against indirect bronchoconstrictor stimuli in asthma is not known. One mechanism could be related to inhibition of sodium transport. If this is the case, then amiloride, another inhibitor of sodium transport, should also protect against indirect bronchoconstrictor challenges.

Ten subjects with mild asthma were administered either 10^{-2} M amiloride or placebo, by nebulizer, in a double-blind crossover fashion. After each inhalation, forced expiratory volume in one second (FEV_1) was recorded at 10 min intervals for 30 min, after which a metabisulphite challenge was performed. No significant difference in the response to metabisulphite was seen between placebo and amiloride treatment. The mean difference in provocative dose of metabisulphite producing a 20% fall in FEV_1 (PD_{20}) between placebo and amiloride was 1.015 doubling doses, 95% confidence interval (95% CI) -0.201 to 2.231, (p=0.09).

This result does not support the hypothesis that frusemide is acting to protect against bronchoconstrictor challenges in asthma by an effect on sodium transport.

Eur Respir J., 1992, 5, 1189–1192.

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Keywords: Amiloride
asthma
metabisulphite

Received: April 8 1992
Accepted after revision July 20 1992

Nebulized frusemide has been shown to protect asthmatic subjects against indirect bronchoconstrictor stimuli including exercise, ultrasonically nebulized distilled water, metabisulphite and the early and late responses to antigen [1–6]. However, frusemide has little or no effect on the response to directly acting bronchoconstrictors, such as histamine or methacholine [5–7]. The mechanism of action of frusemide has not been determined but there are several possibilities to consider.

Frusemide inhibits several ion transport processes including Na/K/Cl co-transport, Na/K adenosine triphosphatase (ATPase) and Cl^-/HCO_3^- exchange, although the latter two are only affected by high concentrations [8]. Other mechanisms of action of frusemide which have been postulated, include inhibition of carbonic anhydrase [9] and increases in prostaglandin E_2 (PGE_2) production [10].

The aim of the present study was to investigate whether the effect of frusemide could be mimicked by amiloride, an inhibitor of Na entry channels and Na/H exchange [11], thus suggesting that frusemide was acting in asthma by modifying transmembrane sodium gradients in airway cells through Na/K/Cl co-transport inhibition. Interestingly, both frusemide and amiloride have been shown to inhibit cough responses to low chloride solutions, suggesting a common mechanism of action [12, 13]. As frusemide has a potent effect on metabisulphite-induced bronchoconstriction [5], we chose this bronchoconstrictor stimulus to study the effect of inhaled amiloride in our study.

Methods

Subjects

We studied 10 nonsmoking men with mild asthma, aged 20–48 yrs (mean 33 yrs). All subjects had a resting forced expiratory volume in one second (FEV_1) >60% of predicted value (range 65–91%) and had previously demonstrated an improvement in FEV_1 of >15% after 200 μg of inhaled salbutamol. They also had a provocative dose of histamine causing a 20% fall in FEV_1 ($PD_{20}FEV_1$) of less than 4 μmol, geometric mean (SEM) 0.58 (0.7) μmol. All subjects were taking inhaled therapy alone, four subjects were taking inhaled steroids regularly (beclomethasone, 200–1,500 μg daily) and all used inhaled β_2-agonists. Inhaled therapy was continued unchanged throughout the study, although β_2-agonists were withheld 6 h

before each study visit. Subjects were excluded if they had an upper respiratory tract infection in the previous four weeks. The study was approved by the Nottingham City Hospital Ethics Committee.

Protocol

The study had a randomized, double-blind, placebo-controlled, crossover design. Subjects attended the laboratory on two non-consecutive days of the same week, at the same time of day, having avoided using inhaled beta-agonists for 6 h prior to each visit. After resting in the sitting position for 30 min, baseline measurements of heart rate, blood pressure and FEV_1 were made, and subjects were asked to inhale, at tidal volume, either 10 ml of 10^{-2} M amiloride in 0.9% sodium chloride or a control solution of 0.9% sodium chloride (osmolarity 308 mosmol·l^{-1}) *via* an Inspiron Mini-neb nebulizer (MMAD 4.7 μ). The concentration of amiloride used was at the limit of its solubility. Heart rate, blood pressure and FEV_1 measurements were repeated at 10 min intervals for 30 min, at which time a sodium metabisulphite challenge test was performed. At the end of each metabisulphite challenge test subjects were given inhaled salbutamol to reverse any bronchoconstriction.

Measurements

FEV_1 was measured as the higher of two readings within 100 ml, using a dry bellows spirometer (Vitalograph, Buckingham, UK. Sodium metabisulphite challenge was performed by a method based on that described by NICHOL *et al.* [5]. Serial dilutions of sodium metabisulphite over the range 0.6–160 mg·ml^{-1}, were made up in normal saline each day. Aerosols were delivered from a nebulizer attached to a breath-actuated dosimeter (MEFAR, Brescia, Italy; output: 95% of particles between 0.5–5 μ); the nebulizer was set to nebulize for 1 s with a pause time of 6 s at a pressure of 22 lb·in^{-2} (152 kPa) and delivered 6.5 μl·$puff^{-1}$. Subjects inhaled doubling doses (0.03–128 μmol) of sodium metabisulphite by inspiring rapidly from functional residual capacity to total lung capacity, holding their breath for 3 s and exhaling slowly for 3 s. FEV_1 was measured 2 min after each dose. The challenge was discontinued when the FEV_1 had fallen by 20% or more, or when subjects had inhaled the highest cumulative dose of sodium metabisulphite (128 μmol). The provocative dose of sodium metabisulphite required to produce a 20% fall in FEV_1 ($PD_{20}FEV_1$MBS) was obtained by interpolation on a log dose response plot.

Analysis

The changes in FEV_1 following amiloride and placebo were compared within subjects by calculating the area under the curve of a plot of FEV_1 against time for each subject. The $PD_{20}FEV_1$MBS values were log transformed for analysis and expressed as geometric mean values. Baseline FEV_1, post-drug FEV_1 and $PD_{20}FEV_1$MBS between amiloride and placebo were compared by paired t-test. The study had 80% power to detect a difference between amiloride and placebo of 1 doubling dose of metabisulphite at the 5% significance level.

Results

Baseline calibre

The mean (SEM) baseline FEV_1 values were 3.54 (0.25) *l* on the placebo study day, and 3.61 (0.27) *l* on the amiloride study day (p=0.266).

Changes in FEV_1 10, 20 and 30 min after administration of placebo or amiloride

There was no significant difference in the area under the curve of FEV_1 between amiloride and placebo (p=0.279). The mean (SEM) of areas under the curve were 106.8 (7.5) *l*·min^{-1} for placebo and 105.0 (7.8) *l*·min^{-1} for amiloride (fig. 1). There was little intersubject variability in FEV_1 responses to amiloride and placebo.

Metabisulphite reactivity values on the two study days

The geometric mean (SEM) of metabisulphite PD_{20} for the placebo study day was 0.58 (0.186) μmol and for the amiloride study day, 1.179 (0.265) μmol. The mean (95% confidence interval) of the difference between amiloride and placebo was 1.015 doubling doses (-0.201 to 2.231) (p=0.09) (fig. 2).

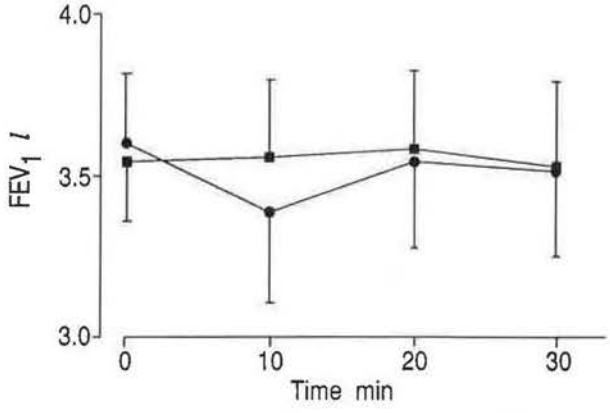

Fig. 1. – Change in forced expiratory volume in one second (FEV_1) after administration of either placebo or amiloride. ■: placebo; ●: amiloride; vertical bars denote SEM.

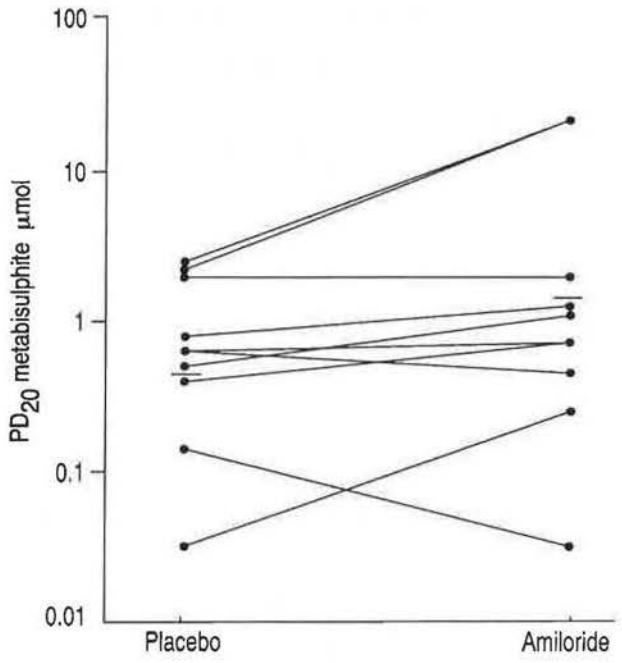

Fig. 2. – Provoking dose of metabisulphite producing a 20% fall in forced expiratory volume in one second (PD_{20}) values for all 10 subjects after placebo and amiloride. ——: geometric mean.

Discussion

The aim of this study was to determine whether the cellular mechanism of action of frusemide in asthma is due to its effect on sodium transport through inhibition of Na/K/Cl co-transport. We therefore studied the effect of another sodium transport inhibitor, amiloride, on metabisulphite-induced bronchoconstriction in a group of 10 subjects with mild asthma. Unlike frusemide, which inhibits Na/K/Cl co-transport [8], amiloride is an inhibitor of Na entry channels and Na/H exchange [11].

We showed no significant change in FEV_1 from baseline after administration of amiloride compared with placebo. Other studies using inhaled amiloride *in vivo* in asthma have also shown no significant effect [9, 14]. This contrasts with the effects of amiloride on airway smooth muscle *in vitro,* which has been studied in both bovine and canine trachealis [15, 16]. Amiloride did not affect the resting tone in bovine tracheal strips [15], but had a marked relaxant effect in strips preconstricted with carbachol. Pretreatment with amiloride (10–1,000 µM) also protected against contraction induced by both histamine and carbachol. KRAMPETZ and BOSE [16] also found that amiloride relaxed canine tracheal strips preconstricted with carbachol and that it had two components, a slow relaxation phase over 16 min and a fast phase of relaxation over 7.5 min. There are several explanations for the discrepancy between the effects of amiloride *in vitro* and *in vivo*. These include differences in species studied, the concentration of drug getting to the target site and the possibility that amiloride is cleared rapidly from the airways *in vivo*. In support of the latter hypothesis, WALTNER *et al.* [17] have shown that the half-life of amiloride in the airway is about 40 min and work in sheep has suggested that amiloride is taken up into the bloodstream very rapidly after inhalation [18].

Sodium metabisulphite is thought to induce bronchoconstriction by release of SO_2, although its precise mechanism of action remains uncertain. Minor degrees of bronchoconstriction seem partly due to a cholinergic reflex [19, 20], but with increasing bronchoconstriction the cholinergic component decreases, so that when metabisulphite aerosols are used to reduce FEV_1 by 20% or more, anticholinergic drugs no longer inhibit the response. The time course of bronchoconstriction and the effects of other drugs on the response to metabisulphite best fit with a neurally-mediated mode of action; the lack of inhibition by anticholinergic drugs suggesting that non-adrenergic, non-cholinergic pathways may be involved [5]. In our study we found that inhaled amiloride did not cause a significant reduction in sensitivity to inhaled metabisulphite compared to placebo. However, there was some variability in responses, with three subjects showing a more marked effect. Whether this reflects real differences in individual responses or measurement variability is debatable. There was no difference in baseline characteristics of these patients from the others. The lack of effect of amiloride contrasts with the inhibitory effect of frusemide on metabisulphite-induced bronchoconstriction in previous studies. It does not support the hypothesis that frusemide is acting in asthma by inhibiting cellular sodium flux in airway cells *via* Na/K/Cl co-transport although the reason for this difference could be pharmacokinetic. Another explanation for the different effects of amiloride and frusemide could be that they are acting through different sodium transport systems, which have different physiological roles. However, studies with bumetamide [21] and torasemide [22], more potent inhibitors of Na/K/C1 co-transport than frusemide, which do not share frusemide's protective properties in asthma, also suggest that co-transport inhibition is not the mechanism of action of frusemide.

The fact that frusemide and amiloride both protect against the cough produced by low chloride solutions [13] suggests that the protective effect of frusemide on cough is mediated *via* a different pathway to its effects on indirect bronchoconstrictor challenges in asthma.

Acknowledgements: The authors thank H. Alexander for secretarial assistance.

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