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# Effect of frusemide on cough responses to chloride-deficient solution in normal and mild asthmatic subjects

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*Effect of frusemide on cough responses to chloride-deficient solution in normal and mild asthmatic subjects. R.A. Stone, P.J. Barnes, K.F. Chung. ©ERS Journals Ltd 1993.*

**ABSTRACT: We studied the tussive effects of a chloride-deficient solution (1.26% sodium bicarbonate).**

**Nine normal volunteers and 10 mild asthmatic subjects were studied. In two double-blind, placebo-controlled, cross-over studies, we assessed the profile of any inhibitory effects that inhaled frusemide had over these responses. Baseline cough challenge was followed by inhalation of either frusemide (40 mg), or 0.15 M NaCl control. Cough was then induced at 0.5, 2, 4 and 6 h after treatment. Forced expiratory volume in one second ($FEV_1$) was measured before and after each challenge. Changes from the baseline cough response due to drug or control were compared nonparametrically at each time point.**

**There was no difference in the sensitivity of normal and asthmatic subjects to the cough challenge (median cough response 15 and 14.5 on control day, 12 and 15 on frusemide day). Frusemide caused sustained inhibition of the cough response in normal subjects ($p<0.05$ at 2 h, $p<0.01$ at 4 h), but had only a small, nonsignificant effect in asthmatic subjects at 30 min. Falls in $FEV_1$ of asthmatic subjects due to the chloride-deficient solution were not significant, and did not correlate with number of coughs.**

**We conclude that mild asthmatic subjects are less sensitive than normal subjects to the influence of frusemide against low chloride challenge. This observation is not explained by bronchoconstrictor effects of the cough challenge in asthmatic subjects.** *Eur Respir J., 1993, 6, 862–867.*

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The loop diuretic frusemide has a number of novel effects within the airway, which include the reduction of cough response to chloride-deficient solutions in normal subjects [1], and the inhibition of several indirect bronchoconstrictor challenges in asthmatic patients [2–6].

The actions of frusemide within the airway are probably multifactorial, but the observation that it affects responses to cough challenge in normals, and to sodium metabisulphite (MBS) in asthmatic subjects has implied an effect on airway nerves. The findings that frusemide reduces neurally-mediated airway smooth muscle contraction *in vitro* [7], and inhibits neural function in a variety of systems outside the lung [8–14], support this assertion. An effect of frusemide on airway nerves, thus, makes the drug potentially useful as a probe to detect differences between airway neural responses of normal and asthmatic subjects, which has been the purpose of the current study.

Asthma is associated with the generation of inflammatory mediators, such as prostaglandins and bradykinin, neuroactive agents that can themselves affect both the function of sensory nerves and the transduction of cough responses [15, 16]. It is, thus, conceivable that cough responses of patients with asthma might differ intrinsically from those of normal subjects in their sensitivity to agents such as frusemide. To investigate this hypothesis, we have examined cough responses of normal and asthmatic subjects to challenge with a chloride-deficient solution, before and after treatment with inhaled frusemide; we have also examined the time course of any inhibitory effects due to frusemide.

## Methods

### *Subjects*

Nine normal non-atopic volunteers, and 10 mild atopic asthmatic subjects (table 1) were studied. All subjects were nonsmokers, and had no history of respiratory infection or cough within the preceding six weeks. Asthmatic subjects were diagnosed on the basis of intermittent symptoms of wheeze and increased airway responsiveness to methacholine (table 1). The asthmatic subjects were using no medication, apart from salbutamol on an "as required basis". None had exacerbation of their asthma within the three months before they enrolled.

## Materials

*Drugs.* Frusemide, (10 $mg \cdot ml^{-1}$ in distilled water containing sodium hydroxide 1.21 $mg \cdot ml^{-1}$, pH 5.3, 209 Mosm; Antigen Ltd, Rosecrea, Ireland) or matched placebo (pH 5.6, 225 Mosm) were diluted in 0.15 M saline [6].

Table 1. – Characteristics of subjects

| Normal subjects | | | | | | | Asthmatic subjects | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | Age yrs | Sex | $FEV_1$ % pred | $PC_{20}$ mg·ml$^{-1}$ | Drugs | Atopy | No. | Age yrs | Sex | $FEV_1$ % pred | $PC_{20}$ mg·ml$^{-1}$ | Drugs* | Atopy** |
| 1 | 30 | M | 110 | >64 | - | - | 1 | 36 | F | 85 | 0.63 | S | + |
| 2 | 27 | F | 100 | >64 | - | - | 2 | 30 | M | 94 | 0.75 | S | + |
| 3 | 24 | F | 98 | >64 | - | - | 3 | 26 | M | 95 | 0.4 | S | + |
| 4 | 29 | F | 95 | >64 | - | - | 4 | 24 | F | 90 | 0.62 | S | + |
| 5 | 23 | F | 101 | >64 | - | - | 5 | 30 | M | 89 | 3.8 | S | + |
| 6 | 38 | M | 99 | >64 | - | - | 6 | 28 | F | 91 | 0.67 | S | + |
| 7 | 20 | F | 104 | >64 | - | - | 7 | 23 | M | 93 | 0.15 | S | + |
| 8 | 24 | M | 106 | >64 | - | - | 8 | 22 | M | 95 | 2.95 | S | + |
| 9 | 23 | F | 95 | >64 | - | - | 9 | 28 | M | 94 | 1.2 | S | + |
| | | | | | | | 10 | 24 | F | 92 | 2.29 | S | + |

$FEV_1$: forced expiratory volume in one second; % pred: percentage predicted; $PC_{20}$: provocative concentration of methacholine producing a 20% fall in $FEV_1$: *: asthmatic subjects used salbutamol (S) only on an as required basis; **: all asthmatic subjects were atopic to common allergens.

A dose of 40 mg frusemide was delivered at the mouth, in a total volume of 10 ml, which was nebulized to dryness from an ultrasonic nebulizer (Ultraneb 99, deVilbiss Ltd, Heston, UK; mass median particle diameter 5 μm). This took 10–15 min.

*Chloride-deficient solution.* Cough was induced by inhalation of a single solution deficient in chloride ions, 0.15 M sodium bicarbonate, pH 8.5, 300 Mosm [1].

### *Protocol*

Studies of identical protocol were completed both in normal volunteers and asthmatic patients. Subjects attended the laboratory on two days, separated by at least 48 h. Asthmatic subjects were not allowed to take any inhaled beta-adrenoreceptor agonist within 4 h of attending the laboratory. In fact, none of the asthmatic subjects had needed to use their inhaler during the 24 h prior to each of the study days.

Subjects arrived at 09.00 h on each study day, and rested for 30 min. A baseline cough challenge was then performed. After 2 h, subjects returned to inhale either frusemide or matched placebo, dispensed in a randomized, double-blind, cross-over fashion. Cough challenge was then repeated at 30 min, 2, 4 and 6 h after treatment with drug or placebo.

All subjects underwent screening prior to entry into the study, to ensure that they coughed, and were familiar with the cough challenge from the outset.

### *Cough challenge*

Sodium bicarbonate, 0.15 M, was chosen as the tussive agent, because we had previously found that cough responses to graded reductions in chloride ion content were maximal and most reproducible with the solution totally deficient in chloride ions [1].

Each subject wore a noseclip, and breathed tidally for one minute through a mouthpiece connected to the same ultrasonic nebulizer containing 15 ml of sodium bicarbonate solution (output 4 mls·min$^{-1}$). The number of coughs was counted during this period, and for a further 1 min after nebulization. No further coughing was noted at later time-points. Coughs were defined as plosive events, occurring singly or in runs. Each plosive event was counted individually, by the same experienced observer (RAS). Episodes of throat clearing, as opposed to coughing, were not counted. $FEV_1$ was recorded on a spirometer (Vitalograph Ltd, Buckingham, UK), before and 1 min after each cough challenge.

### *Data analysis*

Changes in cough response due to the frusemide or placebo were obtained by subtracting the baseline cough response from the cough number at each time point after treatment. These changes were then compared between drug and placebo treatments using Wilcoxon's rank sum analysis. Friedman's analysis was also used to compare changes in cough responses at all time points after frusemide and placebo treatment. Significance was taken as $p<0.05$ for these analyses. The effects of the cough challenge on airway calibre were noted as the percentage difference in $FEV_1$ between values recorded before and after each challenge. Students' paired t-test was used to assess significance, which was again taken as $p<0.05$.

## Results

### *Normal subjects*

In normal volunteers, frusemide reduced the cough response to chloride-deficient challenge. The effect had begun by 30 min, but was not significant until the 2 hr time point ($p<0.05$). It was maximal at 4 h ($p<0.01$), but had approximated to that of placebo by 6 h after treatment. There was also a late attenuation in response following placebo, which was not significant. This may represent either tachyphylaxis to repeated challenge, or a change in sensitivity of the cough reflex during the course of the day. However, this fall did not mask the underlying trend of reduction

caused by frusemide, for the drug effected a different profile of its own. Comparison of all changes in cough response after frusemide against those after placebo was significant ($p<0.01$). Baseline responses on placebo and drug days were not different (medians 15 and 12 coughs respectively).

Figure 1 shows data represented as the change in the cough response from baseline caused by frusemide with respect to placebo. Individual data are shown in table 2, with median cough responses at each challenge point shown in table 3.

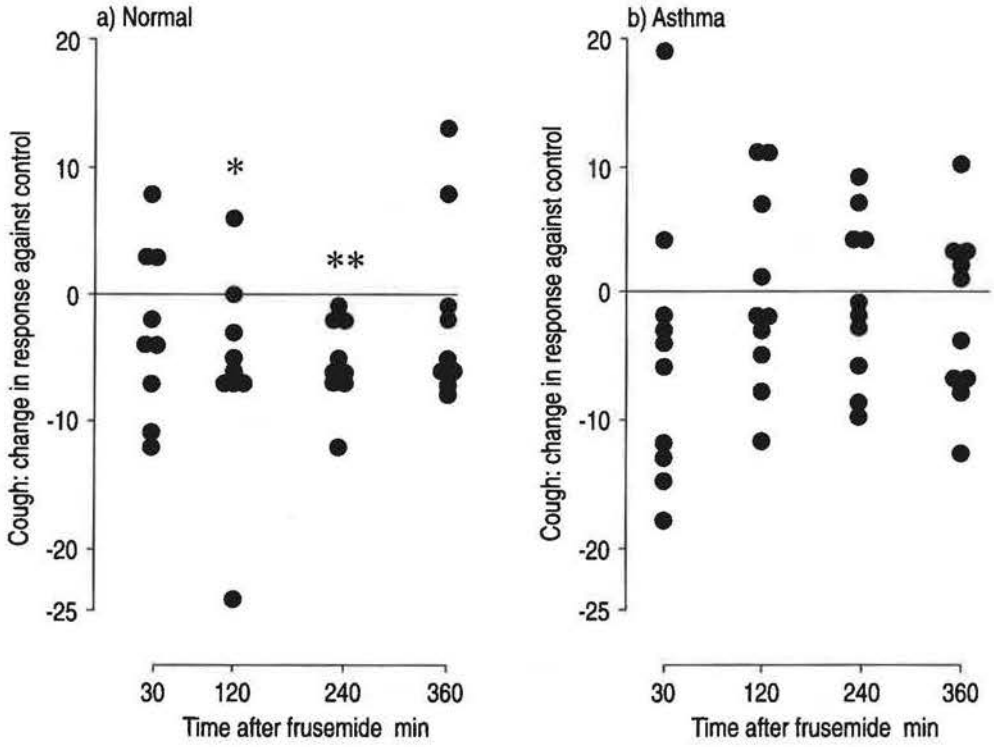


Fig. 1. – Changes in the number of coughs per 2 min at each time point due to frusemide. Data represent the change in cough response from baseline on the frusemide day subtracted from that on the control day. The differences have been compared by Wilcoxon's analysis of paired ranks. There was a significant inhibitory effect of frusemide at 120 (*$p<0.05$) & 240 (**$p<0.01$) min in normal but not asthmatic subjects.

Table 2. – Individual cough responses of normal and asthmatic subjects caused by chloride deficient challenge at each time point before (baseline) and after placebo and frusemide

| No. | BL | Time after placebo min | | | | BL | Time after frusemide min | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 30 | 120 | 240 | 360 | | 30 | 120 | 240 | 360 |
| **Normal** | | | | | | | | | | |
| 1 | 15 | 23 | 22 | 17 | 221 | 12 | 8 | 12 | 12 | 12 |
| 2 | 4 | 6 | 6 | 2 | 2 | 4 | 4 | 0 | 0 | 0 |
| 3 | 24 | 11 | 9 | 9 | 7 | 20 | 3 | 2 | 0 | 11 |
| 4 | 26 | 29 | 31 | 34 | 21 | 20 | 26 | 18 | 27 | 28 |
| 5 | 15 | 8 | 14 | 12 | 5 | 11 | 12 | 16 | 2 | 6 |
| 6 | 10 | 10 | 8 | 8 | 8 | 11 | 4 | 3 | 3 | 3 |
| 7 | 10 | 10 | 12 | 9 | 7 | 12 | 8 | 7 | 4 | 1 |
| 8 | 20 | 17 | 18 | 3 | 8 | 32 | 18 | 6 | 3 | 13 |
| 9 | 14 | 24 | 18 | 22 | 17 | 7 | 20 | 11 | 8 | 9 |
| **Asthma** | | | | | | | | | | |
| 1 | 35 | 55 | 57 | 52 | 41 | 24 | 29 | 34 | 31 | 17 |
| 2 | 20 | 11 | 9 | 17 | 15 | 25 | 14 | 25 | 26 | 22 |
| 3 | 12 | 5 | 14 | 11 | 15 | 6 | 18 | 0 | 14 | 10 |
| 4 | 18 | 12 | 15 | 18 | 17 | 17 | 15 | 21 | 24 | 19 |
| 5 | 3 | 4 | 2 | 2 | 4 | 4 | 1 | 0 | 0 | 1 |
| 6 | 10 | 1 | 6 | 5 | 7 | 17 | 2 | 14 | 6 | 7 |
| 7 | 4 | 13 | 2 | 6 | 6 | 13 | 4 | 6 | 6 | 7 |
| 8 | 17 | 21 | 8 | 11 | 15 | 29 | 21 | 18 | 21 | 20 |
| 9 | 8 | 7 | 1 | 5 | 5 | 4 | 0 | 8 | 5 | 4 |
| 10 | 20 | 11 | 11 | 14 | 10 | 12 | 8 | 1 | 5 | 11 |

BL: baseline

Table 3. – Group median and range of cough responses in normal and asthmatic subjects caused by chloride deficient challenge at each time point on each study day

| | | Time after P or F min | | | |
|---|---|---|---|---|---|
| | BL | 30 | 120 | 240 | 360 |
| **Normals** | | | | | |
| Placebo | 15 (4–26) | 11 (6–29) | 14 (6–31) | 9 (2–34) | 8 (2–21) |
| Frusemide | 12 (4–32) | 8 (3–26) | 7* (0–18) | 3** (0–27) | 9 (0–28) |
| **Asthmatics** | | | | | |
| Placebo | 14.5 (4–35) | 11 (4–55) | 8.5 (1–57) | 11 (2–52) | 12.5 (4–41) |
| Frusemide | 15 (4–29) | 11 (4–29) | 11 (0–34) | 10 (0–31) | 10.5 (1–20) |

Data represent group median and range (in parentheses) of cough frequency per 2 min. Analysis of individual data has been made by comparison of changes from baseline (BL) of placebo (P) and frusemide (F) days using Wilcoxon's analysis of paired ranks. The inhibitory effect of frusemide was observed in normal but not asthmatic subjects at 120 (*$p<0.05$) and 240 (**$p<0.01$) min.

*Asthmatic subjects*

In contrast to its effect on normal volunteers, frusemide had little influence over the cough response in the asthmatic group. Figure 1 shows there is a trend towards activity at 30 min after treatment, with eight subjects demonstrating a reduction in cough response compared to placebo. However, of the nonresponders, one showed considerable potentiation of his cough response at this point on the frusemide day. Moreover, at later time points when frusemide was active in normals, the drug was inactive in asthmatics. Comparison of all changes after frusemide against those after placebo was not significant.

The poor response of asthmatic subjects to frusemide could not be explained by a difference in the sensitivity of each group to the cough challenge, as responses of asthmatic and normal subjects were comparable at baseline attendances on both drug and placebo days (medians 15 and 12 coughs, respectively, for normal subjects, 14.5 and 15 coughs, respectively, for asthmatic subjects). The individual and median cough responses of normals and asthmatics on each study day are shown in tables 2 and 3.

The cough challenge caused a fall in $FEV_1$ in some asthmatic subjects. This fall was not consistent within subjects, and was not significant in the group as a whole. There was no correlation between individual changes in $FEV_1$ (*i.e.* sensitivity to bronchoconstrictor effects of the challenge) and magnitude of cough number on either the frusemide or placebo day of the study (fig. 2). Changes in $FEV_1$ were too inconsistent to demonstrate any influence of frusemide (table 4).

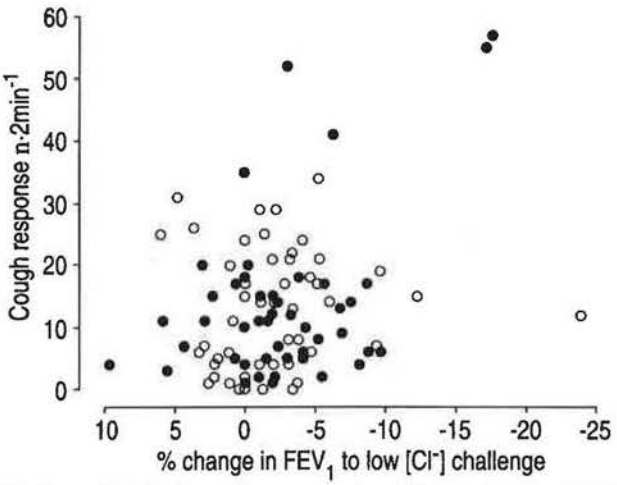


Fig. 2. – Individual cough responses at each time point are plotted against percentage change in $FEV_1$ caused by the chloride-deficient challenge in asthmatic subjects. There is no significant correlation between fall in $FEV_1$ and the cough response. ●: frusemide; ○: control. $FEV_1$: forced expiratory volume in one second.

Table 4. – Group mean change in $FEV_1$ (%) caused by chloride-deficient challenge at each time point in mild asthmatic subjects

| | | Time after P or F min | | | |
|---|---|---|---|---|---|
| | BL | 30 | 120 | 240 | 360 |
| **Placebo** | | | | | |
| Mean | -1.2 | 0.17 | -4.1 | -4.25 | -3.17 |
| SEM | 1.29 | 2.59 | 1.72 | 0.95 | 1.6 |
| **Frusemide** | | | | | |
| Mean | -3.27 | -2 | -1.35 | 0.11 | -2.82 |
| SEM | 2.38 | 0.8 | 1.05 | 1.12 | 1.28 |

For abbreviations see legends to tables 1 and 3.

## Discussion

The data confirm previous observations that frusemide reduces cough response to chloride-deficient challenge in normal volunteers [1]. The effect appears to be sustained, and is maximal 4 h after inhalation of the drug. By contrast, frusemide has no significant effect in asthmatic subjects, although there is a trend towards activity 30 min after treatment.

The slow onset and sustained activity of frusemide against the cough challenge in normal subjects is intriguing. Chloride-deficient solutions stimulate irritant receptors in the larynx of animals [17, 18], but their effects on the distal airway remain unclear. Frusemide has been shown to inhibit chloride transport and a number of other processes controlling neural excitability [8–14]. However, there are no single-fibre studies assessing effects of

frusemide on airway nerves or sensory receptors in the lung. The drug has been used in cochlear nerve preparations, where it has similarly been observed to cause sustained inhibition of acoustic responses, that are maximal 26 min after application, but still significant 100 min later [19]. The mechanism of this effect is unknown. Chloride-deficient solutions also recruit myelinated cochlear nerve fibres at equivalent anion concentrations to those that excite airway nerves [12].

The inactivity of frusemide against the chloride-deficient solution in asthmatic subjects could not be explained by differences in the sensitivity of their cough reflex, or by bronchoconstrictor effects of the challenge; baseline cough responses of each group were similar, as noted by others [20–23], and $FEV_1$ was unaffected by inhalation of the chloride-deficient solution, as also noted by others [24]. Differences in the deposition of frusemide between normal and asthmatic subjects could have occurred, but this is unlikely because the pattern of aerosol deposition in the airways of asthmatics with $FEV_1$ within the normal range is the same as that for normal subjects [25].

The data, therefore, suggest that sensory nerves subserving cough induced by chloride-deficient solutions in asthmatic subjects may be intrinsically less sensitive to the inhibitory effects of frusemide. The reason for this remains speculative. It is possible that the products of airway inflammation in asthmatic subjects could alter the sensitivity of airway receptors subserving cough in this group, hence leading to reduced effects of frusemide; increased levels of inflammatory mediators and cytokines have been detected in the bronchoalveolar lavage fluid of asthmatic patients [26, 27] and, although the effects of these mediators and cytokines on airway sensory nerves are not well-studied, it is known that, outside the lung, cytokines can modulate peptide content of nerves [28], opioid receptors [29], nerve growth [30], and nerve conduction [31].

Our studies also highlight differences in the effects of frusemide against bronchoconstrictor and tussive stimuli in patients with mild asthma. Whilst frusemide influences a number of bronchoconstrictor challenges *via* effects which may involve the airway epithelium and inflammatory cells [2–6], it is clear that cough and bronchcoconstrictor reflexes may be mediated through different afferent neural pathways [20, 24]. The inhibition of bronchoconstriction need not necessarily imply the inhibition of cough [20].

In summary, we have shown that frusemide has sustained effects against chloride-deficient cough challenge in normals, but is relatively inactive in asthmatic subjects. This may reflect reduced sensitivity to frusemide of airway nerves subserving this cough response in asthmatic subjects.

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