Eur Respir J, 1995, 8, 593–599
DOI: 10.1183/09031936.95.08040593
Printed in UK - all rights reserved


European Respiratory Journal
ISSN 0903 - 1936

# Inhaled loop diuretics and basal airway responsiveness in man: evidence of a role for cyclo-oxygenase products

R. Polosa*, K. Rajakulasingam**, G. Prosperini*, S. Magrì*, C. Mastruzzo*, S.T. Holgate**

*Inhaled loop diuretics and basal airway responsiveness in man: evidence of a role for cyclo-oxygenase products. R. Polosa, K. Rajakulasingam, G. Prosperini, S. Magrì, C. Mastruzzo, S.T. Holgate. ERS Journals Ltd 1995.*

**ABSTRACT: Inhaled frusemide protects asthmatic airways against a wide variety of bronchoconstrictor stimuli by unknown mechanisms.**

**To investigate whether inhaled loop diuretics modulate baseline bronchial responsiveness, a randomized, double-blind, placebo-controlled study was conducted to test the ability of frusemide (40 mg) and bumetanide (2 mg) to displace concentration-response curves with methacholine in 14 healthy volunteers. In addition, separate randomized, double-blind studies were carried out to evaluate the effects of oral flurbiprofen, a potent cyclo-oxygenase inhibitor, on the protective action of frusemide against methacholine-induced bronchoconstriction.**

**Inhaled loop diuretics significantly increased the provocative concentration of methacholine causing a 15% decrease in forced expiratory volume in one second ($PC_{15}FEV_1$) from the geometric mean (range) value of 58.6 (9.2–233) mg·ml$^{-1}$ after placebo administration, to 129 (13.8–505) and to 106 (6.6–510) mg·ml$^{-1}$ after administration of frusemide and bumetanide, respectively. Similar results were obtained when data from partial flow-volume curves were used for analysis. In the eight subjects studied, pretreatment with oral placebo and inhaled frusemide reduced airway responsiveness to methacholine, with a geometric mean (range) $PC_{15}FEV_1$ value of 116 (25.4–405) mg·ml$^{-1}$, and premedication with oral flurbiprofen abolished this protective effect, the geometric mean (range) $PC_{15}FEV_1$ methacholine being reduced to a value of 50.3 (16.6–189) mg·ml$^{-1}$. In addition, oral flurbiprofen alone failed to alter airway responsiveness to methacholine.**

**In view of these findings, it is suggested that bronchoprotective prostaglandins may mediate the effects of loop diuretics against methacholine-induced bronchoconstriction in man.**

*Eur Respir J., 1995, 8, 593–599.*

*Istituto Malattie Apparato Respiratorio, Università di Catania, Catania, Italy. **Medicine 1, Southampton General Hospital, Southampton, UK.

Correspondence: R. Polosa
Istituto Malattie Apparato Respiratorio
Università di Catania
Via Passo Gravina 187
95125 Catania
Italy

Keywords: Bumetanide
frusemide
humans
methacholine challenge

Received: June 15 1994
Accepted after revision January 2 1995

When administered by inhalation, the "loop" diuretic frusemide has been shown to protect the asthmatic airways against various bronchoconstrictor stimuli, including exercise [1], ultrasonically nebulized distilled water [2], allergen [3], adenosine 5'-monophosphate [4], sodium metabisulphite [5], and bradykinin [6]. However, it has little or no effect on the bronchoconstriction following directly acting agonists, such as methacholine [4, 5] and histamine [7].

The mechanisms underlying the protective effects of frusemide in asthma are not yet understood, but are of interest as they might shed light on the pathophysiology of asthma [8]. In common with other loop diuretics, frusemide and bumetanide act on the renal tubule to promote diuresis by inhibiting the $Na^+2Cl^-K^+$ co-transporter on the luminal surface of the epithelia [9]. If this is true for the airway epithelium, then exposure to a loop diuretic might alter local neural activity or inflammatory cell activation *via* a change in the osmolarity of the surrounding airway lining fluid. However, the lack of effect of frusemide when administered orally argues against this mechanism [1, 3], as does the evidence that bumetadine, one of the most potent inhibitors of the $Na^+2Cl^-K^+$ co-transporter, is appreciably less efficacious than equidiuretic doses of frusemide in its ability to protect against the bronchoconstriction induced by different stimuli in asthmatic subjects [10, 11]. An additional possibility is that bronchoprotective prostaglandins, such as prostaglandin $E_2$ ($PGE_2$) and prostacyclin ($PGI_2$), may underlie the airway action of loop diuretics in asthma. In man, frusemide enhances the synthesis of $PGE_2$ in the kidney, which may affect renal blood flow [12, 13]. That this also occurs in the airways is supported by a recent report showing that bovine tracheal mucosa produces $PGE_2$ in response to frusemide [14]. As inhaled $PGE_2$ has been shown to protect against the bronchospastic response to methacholine, sodium metabisulphite, exercise and allergen [15–17], the beneficial effects of inhaled frusemide may be related to local airway production of $PGE_2$.

Although it has been reported that inhaled frusemide may reduce baseline bronchial responsiveness in man [18], it is not known whether a similar protective effect is seen with other loop diuretics. We have, therefore, investigated the effects of inhaled frusemide and bumetanide on airway responsiveness to methacholine in a group of healthy volunteers. In addition, we have tested the hypothesis that this phenomenon may be due to generation of cyclo-oxygenase products in the airway by investigating the effect of flurbiprofen, a potent cyclo-oxygenase inhibitor, on the protective action of inhaled frusemide against methacholine-induced bronchoconstriction. As part of these studies, the effect of flurbiprofen on airway responsiveness to methacholine was also examined in a separate randomized, double-blind study.

## Methods

### *Subjects*

A total of 22 nonsmoking healthy subjects (12 males and 10 females) aged 18–55 yrs, with no history of respiratory disease and with baseline values of forced expiratory volume in one second ($FEV_1$) above 80% of their maximum predicted values, took part in the study (table 1). Sixteen subjects were atopic, as defined by positive responses to skin-prick tests (weals over 3 mm in diameter) to one or more common aeroallergens. The subjects took no medication and were free from respiratory tract infections for at least 4 weeks before and throughout the investigation. None gave a history of nonsteroidal anti-inflammatory drug (NSAID) intolerance or developed bronchoconstriction in response to oral flurbiprofen. Subjects gave their written informed consent, and the study was approved by the Southampton University and Hospital Ethics Committee.

Table 1. – Demographic details of subjects studied

| Sub No. | Sex | Age yrs | Baseline $FEV_1$ *l* | Atopy§ | $PC_{15}$ meth mg·ml$^{-1}$ |
|---|---|---|---|---|---|
| 1 | F | 21 | 3.76 | + | 34.0 |
| 2 | F | 26 | 3.02 | + | 60.5 |
| 3 | M | 28 | 3.10 | + | 39.1 |
| 4 | M | 29 | 4.57 | + | 28.6 |
| 5 | M | 23 | 3.91 | - | 43.8 |
| 6 | M | 23 | 3.65 | + | 204.5 |
| 7 | M | 22 | 3.75 | + | 117.2 |
| 8 | F | 55 | 2.26 | - | 225.1 |
| 9 | F | 24 | 3.07 | + | 61.3 |
| 10 | M | 34 | 2.67 | + | 8.3 |
| 11 | M | 21 | 3.70 | - | >512 |
| 12 | M | 36 | 3.97 | + | >512 |
| 13 | M | 21 | 5.03 | + | >512 |
| 14 | M | 22 | 4.35 | + | >512 |
| 15 | F | 26 | 3.10 | + | 10.7 |
| 16 | F | 30 | 2.95 | - | 18.5 |
| 17 | M | 28 | 3.75 | + | 25.5 |
| 18 | M | 33 | 3.85 | + | 150.9 |
| 19 | F | 18 | 3.20 | + | 112.2 |
| 20 | F | 44 | 2.82 | - | 92.5 |
| 21 | F | 31 | 2.97 | + | 88.8 |
| 22 | F | 30 | 3.08 | - | 147.1 |
| Mean | | 28 | 3.48 | | 83.6* |
| SEM | | ±2 | ±0.14 | | (8.3–>512) |

*: geometric mean and range in parenthesis; §: atopic, positive (+) immediate skin-prick test to one or more allergens. $FEV_1$: forced expiratory volume in one second; $PC_{15}$ meth: provocation concentration of methacholine producing a 15% decrease in $FEV_1$; Sub: subject; M: male; F: female.

### *Airway measurements and methacholine bronchoprovocation tests*

Airway calibre was recorded both as the $FEV_1$ and as the maximum expiratory flow rate measured at 70% of the vital capacity (VC) below total lung capacity (TLC) from a partial forced expiratory manoeuvre ($\dot{V}p_{30}$). Both measurements were derived from flow-volume curves produced on a rolling seal, flow-rate-dependent spirometer (Morgan Spiroflow, P.K. Morgan Ltd, Kent, UK) connected to an 85B desk top computer *via* an 82940A GP-10 interface (Hewlett Packard, Wokingham, Berkshire, UK). Partial expiratory flow-volume (PEFV) curves were obtained using the technique described by ZAMEL [19]. Briefly, three VC measurements were recorded, and the largest used to define the control VC. The volume from which the PEFV curves were initiated was set at 70% of this control VC and marked-off from TLC. After at least one minute of tidal breathing, during which deep breaths were carefully avoided, subjects were asked to reach, from end-tidal expiration, a volume equal to the 70% of their control VC, and to expire forcefully to residual volume (RV). In this way, PEFV curves, standardized for volume and volume history, were obtained and related $\dot{V}p_{30}$ values derived. On reaching RV, subjects inspired to TLC and then expired maximally back to RV, allowing a measurement of $FEV_1$ to be recorded.

Methacholine (Sigma Chemical Co., Poole, Dorset, UK) was prepared in 0.9% sodium chloride (saline) to produce a range of doubling concentrations of 1–256 mg·ml$^{-1}$ (5.1–1308 mmol·*l*$^{-1}$). The aqueous solutions were administered as aerosols, generated from a starting volume of 3 ml in a disposable Inspiron Mini-nebulizer (C.R. Bard International, Sunderland, UK) driven by compressed air at 8 *l*·min$^{-1}$. Subjects inhaled the aerosolized solutions in five breaths from end-tidal volume to full inspiratory capacity *via* a mouthpiece. Subjects were trained to take 3 s to reach full inspiratory capacity.

### *Study design*

The study consisted of three separate phases.

*Phase 1.* Fourteen subjects (Nos. 1–14) attended the laboratory on four separate occasions, at least 72 h apart, at the same time of day.

On the initial visit, subjects undertook a concentration-response study with inhaled methacholine in the absence of any drug treatment. After 15 min rest, three baseline

measurements of $FEV_1$ and $\dot{V}p30$ were made at intervals of 3 min, followed by inhalation of saline and further measurements repeated at 1 and 3 min, the higher values being recorded. A concentration-response study with methacholine was then carried out. After administration of each concentration, $FEV_1$ and $\dot{V}p30$ were measured at 1 and 3 min. Increasing doubling concentrations of methacholine were inhaled at approximately 5 min intervals until $FEV_1$ had fallen by >15% of the post-saline value and the corresponding $PC15FEV_1$ values derived, or until $\dot{V}p30$ had decreased by >40% and the related $PC40\dot{V}p30$ values calculated.

On the next three visits, subjects underwent concentration-response studies with inhaled methacholine after nebulized frusemide (40 mg), bumetanide (2 mg) or matched nebulized vehicle (placebo), administered in double-blind fashion and in random order 10 min before challenge. On each occasion, three baseline measurements of $FEV_1$ and $\dot{V}p30$ were recorded at intervals of 3 min. This was followed by inhalation of nebulized frusemide (Lasix, Hoechst, Frankfurt AM Main, Germany) at a concentration of 10 $mg \cdot ml^{-1}$, nebulized bumetanide (Burinex, Leo, Aylesbury, UK) at a concentration of 0.50 $mg \cdot ml^{-1}$, or nebulized vehicle consisting of 0.9% sodium chloride adjusted to similar pH and tonicity as the drug solution used. The aerosol solutions were generated from a starting volume of 4.0 ml in an Inspiron mini-nebulizer, driven by compressed air at a rate of 6 $l \cdot min^{-1}$, and inhaled to dryness by deep tidal breathing over a 7–9 min time period. The same nebulizer was used for all studies on all subjects. $FEV_1$ and $\dot{V}p30$ measurements were taken 5 and 10 min after inhalation. Ten minutes after inhalation of the test drug, a concentration-response study with methacholine was performed in the manner described above.

*Phase 2.* Eight subjects (Nos. 1–4, 6, 7, 15 and 16) with a previously established $PC15FEV_1$ methacholine attended the laboratory on two separate occasions at the same time of day, not less than 10 days apart.

On each occasion, subjects were asked to take oral flurbiprofen, 50 mg *b.i.d.* or matched placebo for 3 days before and on the day of the challenge, according to a randomized double-blind protocol, the last dose being taken 2 h before the methacholine challenge. On both visits, 10 min before the challenge, subjects inhaled frusemide (40 mg) through a mouthpiece at tidal volume until the nebulizer was dry. $FEV_1$ measurements were taken before, and 5 and 10 min after inhalation. The administration of the aerosol solutions and the challenge procedure used were identical to those described in Phase 1 of the study.

*Phase 3.* Eight subjects (Nos. 15–22) with a previously established $PC15FEV_1$ methacholine attended the laboratory on two further occasions at the same time of day.

On each occasion, subjects were asked to take oral flurbiprofen, 50 mg *b.i.d.* or matched placebo for 3 days before and on the day of the challenge, according to a randomized double-blind protocol, the last dose being taken 2 h before the methacholine challenge. On both visits, 10 min before the challenge, subjects inhaled placebo (normal saline) followed by $FEV_1$ measurements and methacholine challenge in a manner similar to that described for Phase 2 of the study.

### *Data analyses*

Results are expressed as mean±SEM unless otherwise stated, and a p value of less than 0.05 was accepted as the minimum level of statistical significance. Pre- and post-treatment baseline values of $FEV_1$ and $\dot{V}p30$ prior to bronchial challenges were compared between and within study days using analysis of variance (ANOVA) for multiple comparisons, and the change assessed by the least significant difference test.

Concentration-response curves were constructed by plotting the percentage change in $FEV_1$ and $\dot{V}p30$ from the postsaline baseline value against the cumulative concentration of methacholine administered on a logarithmic scale and the concentration of agonist required to produce a 15 and 40% fall in $FEV_1$ and in $\dot{V}p30$ from the postsaline baseline value ($PC15FEV_1$ and $PC40\dot{V}p30$, respectively) determined by linear interpolation.

Values of $PC15FEV_1$ and $PC40\dot{V}p30$ methacholine following treatments were logarithmically transformed to normalize their distribution, and compared by ANOVA for multiple comparisons (for Phase 1) and by Student's t-test for paired data (for Phases 2 and 3).

In Phase 1, four subjects (Nos. 11–14) failed to achieve a 15% fall in $FEV_1$ when the maximum concentration of methacholine was administered and their minimal estimated values were excluded from statistical analysis.

For each subject, concentration ratios for the protective effect of frusemide and bumetanide against bronchoprovocation with methacholine were calculated individually, by dividing the PC values obtained after administration of active drug by that obtained after placebo, and compared using the Wilcoxon signed rank test.

In addition, we have analysed any relationship between the concentration ratios after the test drugs, the baseline $FEV_1$ and the airway responses to methacholine by least-squares linear regression analysis.

## Results

*Phase 1.* There were no significant differences in mean baseline values of $FEV_1$ and $\dot{V}p30$ between any of the treatment days. Inhaled frusemide and bumetanide were well-tolerated and had no effect on baseline spirometry, with mean±SEM values ranging from 3.57 (±0.21) to 3.67 (±0.20) $l$ for $FEV_1$, and from 161.5 (±13.3) to 169.0 (±13.1) $l \cdot min^{-1}$ for $\dot{V}p30$. Subjects Nos. 11–14 did not achieve a 15% fall in $FEV_1$ after methacholine challenge on any of the study days, and their minimal estimated values were excluded from statistical analysis.

On the placebo study day, the geometric mean (range) PC15 (n=10) and PC40 (n=14) values with methacholine in this group of subjects were found to be 58.6 (9.2–233.0) and 14.8 (1.7–69.8) $mg \cdot ml^{-1}$ respectively, which did not

differ significantly from the $PC_{15}$ and $PC_{40}$ values of 55.5 (6.5–225.1) and 13.1 (1.2–50.5) mg·ml$^{-1}$ obtained on the open study day.

When compared with placebo, both frusemide and bumetanide protected the airways against the constrictor effects of inhaled methacholine. When airway responsiveness was analysed as $PC_{15}$ (n=10), frusemide produced a displacement of the methacholine concentration-response curves to the right in 9 out of the 10 subjects, whereas protection with bumetanide was observed in only 7 subjects (table 2a).

Inhaled frusemide had a significant protective effect against the fall in $FEV_1$ and $\dot{V}p_{30}$ provoked by methacholine (figs. 1 and 2; table 2a and b), the geometric mean (range) $PC_{15}$ and $PC_{40}$ values increasing from 58.6 and 14.8 mg·ml$^{-1}$ after placebo administration to 129 (13.8–505) mg·ml$^{-1}$ ($p<0.01$) and to 32.2 (1.6–182) mg·ml$^{-1}$ ($p<0.01$) after administration of the drug. Inhaled bumetanide was as effective as frusemide in protecting against the methacholine-provoked fall in $FEV_1$ and $\dot{V}p_{30}$ (figs. 1 and 2; table 2a and b); the geometric mean $PC_{15}$ and $PC_{40}$ values increased to 106 (6.6–510) mg·ml$^{-1}$ ($p<0.05$) and to 24.0 (1.3–202) mg·ml$^{-1}$ ($p<0.05$).

Table 2. – Effects of inhaled bumetanide, frusemide and placebo on airway methacholine responsiveness measured as $PC_{15}FEV_1$ and $PC_{40}\dot{V}p_{30}$

| Subject No. | Placebo mg·ml$^{-1}$ | Bumetanide mg·ml$^{-1}$ | Frusemide mg·ml$^{-1}$ |
|---|---|---|---|
| **a) $PC_{15}FEV_1$ methacholine** | | | |
| 1 | 37.9 | 92.2 | 63.0 |
| 2 | 37.2 | 73.0 | 50.7 |
| 3 | 55.5 | 110.5 | 146.4 |
| 4 | 30.5 | 82.0 | 338.5 |
| 5 | 52.1 | 249.2 | 71.6 |
| 6 | 116.0 | 119.5 | 306.3 |
| 7 | 155.7 | 510.3 | 505.0 |
| 8 | 233.0 | 355.6 | 500.7 |
| 9 | 100.1 | 83.2 | 102.2 |
| 10 | 9.2 | 6.6 | 13.8 |
| 11 | >512 | >512 | >512 |
| 12 | >512 | >512 | >512 |
| 13 | >512 | >512 | >512 |
| 14 | >512 | >512 | >512 |
| GM | 58.6 | 106.1 | 129.0 |
| Range | (9.2–233.0) | (6.6–510.3) | (13.8–505.0) |
| **b) $PC_{40}\dot{V}p_{30}$ methacholine** | | | |
| 1 | 13.9 | 22.4 | 16.7 |
| 2 | 16.2 | 14.3 | 8.2 |
| 3 | 30.8 | 91.1 | 108.4 |
| 4 | 18.0 | 38.9 | 137.5 |
| 5 | 8.7 | 36.2 | 10.2 |
| 6 | 23.5 | 44.9 | 37.8 |
| 7 | 4.5 | 28.3 | 94.9 |
| 8 | 50.8 | 30.4 | 64.5 |
| 9 | 30.2 | 17.8 | 36.9 |
| 10 | 1.7 | 1.3 | 1.6 |
| 11 | 2.4 | 3.0 | 8.7 |
| 12 | 11.4 | 16.5 | 41.0 |
| 13 | 42.6 | 201.9 | 69.4 |
| 14 | 69.8 | 57.1 | 181.7 |
| GM | 14.8 | 24.0 | 32.2 |
| Range | (1.7–69.8) | (1.3–201.9) | (1.6–181.7) |

*: geometric mean values calculated excluding subjects Nos 11–14 from analysis. $PC_{40}\dot{V}p_{30}$: provocation concentration of methacholine producing a 40% decrease in $\dot{V}p_{30}$; $\dot{V}p_{30}$: maximum expiratory flow rate measured at 70% of vital capacity below total lung capacity from a partial forced expiratory manoeuvre. For further abbreviations see legend to table 1.

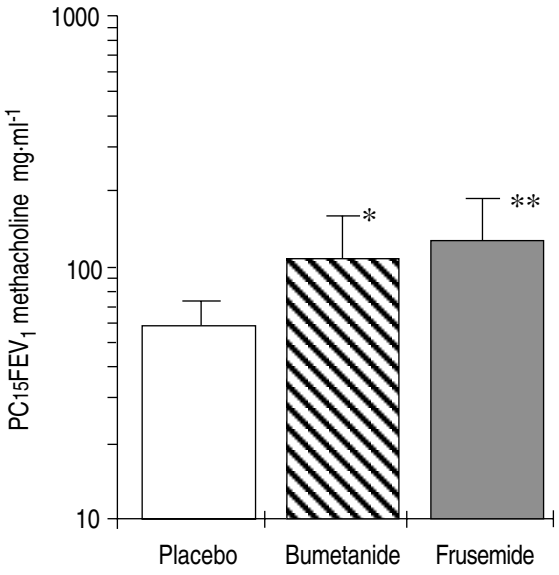


Fig. 1. – Changes (mean±SEM) in provocation concentrations of methacholine required to provoke a 15% decrease in $FEV_1$ ($PC_{15}FEV_1$) after administration of placebo (□), frusemide (■) and bumetanide (▨) in 10 normal subjects. *: significant difference at $p<0.05$ *vs* placebo; **: significant difference at $p<0.01$ *vs* placebo. $FEV_1$: forced expiratory volume in one second.

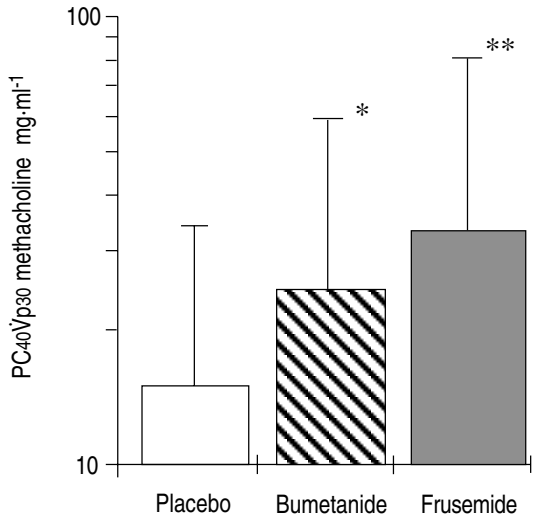


Fig. 2. – Changes (mean±SEM) in provocation concentrations of methacholine required to provoke a 40% decrease in $\dot{V}p_{30}$ ($PC_{40}\dot{V}p_{30}$) after administration of placebo (□), frusemide (■) and bumetanide (▨) in 14 normal subjects. *: significant difference at $p<0.05$ *vs* placebo. **: significant difference at $p<0.01$ *vs* placebo. $\dot{V}p_{30}$: maximum expiratory flow rate measured at 70% of vital capacity below total lung capacity from a partial forced expiratory manoeuvre.

When expressed as concentration ratios of PC15 data, frusemide afforded a 2.2 fold protection of the airways against methacholine, whereas the protective effect of bumetanide was 1.8 fold. Similar findings were obtained when the concentration ratios for the PC40 values were used for analysis. No significant relationship could be found between the concentration ratios after the test drugs, and the airway responses to methacholine.

*Phase 2.* There were no significant differences in mean baseline values of $FEV_1$ between the two study days. Inhaled frusemide alone or in combination with oral flurbiprofen had no effect on baseline spirometry.

As expected, pretreatment with oral placebo and inhaled frusemide reduced airway responsiveness to methacholine, with a geometric mean (range) PC15 value of 116 (25.4–405) mg·ml$^{-1}$, and combined premedication with oral flurbiprofen and inhaled frusemide abolished this protective effect, the geometric mean (range) $PC_{15}FEV_1$ methacholine being reduced to a value of 50.3 (16.6–189) mg·ml$^{-1}$ (table 3).

*Phase 3.* There were no significant differences in mean baseline values of $FEV_1$ between the two study days. Oral flurbiprofen had no effect on baseline spirometry.

Table 3. – Individual $PC_{15}FEV_1$ methacholine values after inhaled frusemide in combination with oral flurbiprofen or matched placebo pretreatment (Phase 2)

| | $PC_{15}FEV_1$ methacholine mg·ml$^{-1}$ | |
|---|---|---|
| Subject No. | Placebo + frusemide | Flurbiprofen + frusemide |
| 1 | 57.7 | 32.3 |
| 2 | 69.0 | 41.1 |
| 3 | 168.8 | 50.0 |
| 4 | 291.6 | 67.9 |
| 6 | 405.2 | 188.8 |
| 7 | 399.4 | 155.0 |
| 15 | 41.4 | 18.7 |
| 16 | 25.4 | 16.6 |
| GM | 116.3 | 50.3 |
| range | (25.4–405.2) | (16.6–188.8) |

For abbreviations see legend to table 1.

Table 4. – Individual $PC_{15}FEV_1$ methacholine values after inhaled normal saline in combination with oral flurbiprofen or matched placebo pretreatment (Phase 3)

| | $PC_{15}FEV_1$ methacholine mg·ml$^{-1}$ | |
|---|---|---|
| Subject No. | Placebo + saline | Flurbiprofen + saline |
| 15 | 16.6 | 28.0 |
| 16 | 10.3 | 9.4 |
| 17 | 33.9 | 42.2 |
| 18 | 187.3 | 170.0 |
| 19 | 98.0 | 88.8 |
| 20 | 105.2 | 119.0 |
| 21 | 64.8 | 87.2 |
| 22 | 169.1 | 191.5 |
| GM | 57.7 | 65.4 |
| Range | (10.3–187.3) | (9.4–191.5) |

Pretreatment with oral flurbiprofen and inhaled placebo failed to alter airway responsiveness to methacholine, the geometric mean (range) PC15 value of 65.4 (9.4–192) mg·ml$^{-1}$ not being significantly different from that of 57.7 (10.3–187) mg·ml$^{-1}$ obtained after placebos (table 4).

## Discussion

In the present study, we have shown that loop diuretics attenuate methacholine-induced bronchoconstriction in man. The approximately twofold reduction in the airway response to methacholine after inhaled frusemide and bumetanide is in agreement with the findings of an open investigation by FUJIMURA *et al.* [18] on 11 healthy subjects, and with the results of a previous investigation of 12 asthmatics by our group [4]. These findings indicate that inhaled loop diuretics may cause a reduction in the level of nonspecific bronchial responsiveness in man.

The present data differ from those of others obtained in asthmatic subjects [5, 10]. Clearly, there must be something in the characteristics of normal subjects which affects their response, as indicated by a recent study [20] in which inhaled frusemide caused sustained inhibition of the cough response to nebulized low-chloride solutions in normals, but not in asthmatics. Damaged airway epithelium in asthma may alter the pharmacokinetic profile of inhaled loop diuretics, thus, changing their local pharmacodynamic activity. Therefore, it is possible that local drug concentrations may persist at higher levels in the airways of healthy subjects, as opposed to asthmatics, so that a more significant response is seen. In addition, the damaged airway epithelium in asthma might respond differently to loop diuretics by generating less bronchoprotective prostaglandins (*i.e.* $PGE_2$), as shown for viral infections [21].

We have also shown that pretreatment with oral flurbiprofen for 3 days, at a dose that abolished *ex vivo* production of thromboxane metabolites [22], abolished the effect of frusemide in protecting against methacholine-induced bronchoconstriction. This reversal of the protective effects of frusemide by a cyclo-oxygenase inhibitor provides strong evidence for our hypothesis that the beneficial effects of frusemide in man are due to production of inhibitory prostanoids. This view is also supported by a recent study by PAVORD *et al.* [23], in which pretreatment with the potent cyclo-oxygenase blocker, indomethacin, elicited a significant reduction of the effect of frusemide in protecting against exercise-induced asthma. However, in a recent study of similar design, another cyclo-oxygenase inhibitor (flurbiprofen, 200 mg in a single oral dose), failed to reverse the protective effect of inhaled frusemide against metabisulphite (MBS)-induced bronchoconstriction in asthmatics [24]. We have no convincing explanation for these discrepancies, but it should be borne in mind that different dosages of flurbiprofen were used. In addition, the possibility of a decrease in $PGE_2$ production by the asthmatic epithelium may be responsible for the observed failure of oral flurbiprofen to reverse the protective effect of inhaled frusemide against MBS-induced bronchoconstriction in asthma.

Both $PGE_2$ and prostacyclin ($PGI_2$) are major cyclo-oxygenase products of human lung [25, 26], and loop diuretics have been shown to induce their effect in the kidney by the secondary production of $PGE_2$ and $PGI_2$ [12, 13, 27]. In man, frusemide stimulates the production of $PGE_2$ by increasing the availability for arachidonic acid [28], and enhances the urinary excretion of $PGI_2$ [29]. Although there is, as yet, no direct evidence to implicate frusemide in the production of inhibitory prostaglandins from human epithelium *in vivo*, it is suggested that frusemide may afford protection against a variety of stimuli by releasing epithelium derived $PGE_2$ and $PGI_2$, both of which are potent functional antagonists through their capacity to stimulate adenylate cyclase in the airways [30]. Indeed, $PGE_2$ has inhibitory effects on cholinergic contractions of human airway preparations after electric field stimulation [31], and inhaled $PGE_2$ protects against the bronchoconstrictor response to methacholine, sodium metabisulphite, exercise, ultrasonically-nebulized distilled water (UNDW) and allergen in asthmatic subjects [15–17]. Similarly, inhaled $PGI_2$ affords short-term protection against several stimuli including UNDW [32], $PGD_2$ [33] and methacholine [33], without having any consistent effect on basal airway calibre. Studies with cyclo-oxygenase inhibitors corroborate a protective role for endogenous inhibitory prostaglandins, by showing a reduction in the loss of responsiveness observed after recovery from bronchoconstriction induced by exercise [34] and UNDW [35].

Although both flurbiprofen and frusemide are weak organic acids and may compete for a similar transport system across the airway epithelium, it is unlikely that the findings of the present study might have been explained on grounds other than inhibition of prostaglandin synthesis. This would have been important if both drugs were to be administered by inhalation. Although local production of inhibitory prostaglandins may be considered as an important mechanism by which inhaled loop diuretics protect the airways against bronchoconstriction, additional possibilities include the suppressive effect on the function of airway mast cells [36], and the capacity of this drug to inhibit neural pathways [37, 38].

In the subjects studied, flurbiprofen alone did not inhibit the bronchoconstrictor response to methacholine. This finding was not surprising, as a number of studies with this drug have repeatedly failed to show an effect on methacholine or histamine induced bronchoconstriction [39, 40].

The present study demonstrates that methacholine-induced bronchoconstriction is inhibited both by inhaled frusemide and bumetanide in man, and that the protective effect of frusemide is reversed by cyclo-oxygenase blockade, thus supporting the view that the beneficial effects of frusemide in human airways are due to the local production of inhibitory prostaglandins. Although our work suggests that functional antagonisms sustained by inhibitory prostaglandins, such as $PGE_2$ and $PGI_2$ may offer an additional explanation for many of the protective effects of loop diuretics in asthma, further research is required to elucidate the suppressive action of this class of drug on bronchospasm and to explore how these findings may broaden our understanding of the potential role of these compounds in asthma.

## References


1. Bianco S, Vaghi A, Robuschi M, Pasargiklian M. Prevention of exercise-induced bronchoconstriction by inhaled frusemide. *Lancet* 1988; ii: 252–255.
2. Robuschi M, Gambard G, Spagnotto S, Vaghi A, Bianco S. Inhaled frusemide is highly effective in preventing ultrasonically nebulised water bronchoconstriction. *Pulm Pharmacol* 1989; 1: 187–191.
3. Bianco S, Pierono MG, Refini RM, Rottoli L, Sestini P. Protective effect of inhaled frusemide on allergen-induced early and late asthmatic reactions. *N Engl J Med* 1989; 321: 1069–1073.
4. Polosa R, Lau LCK, Holgate ST. Inhibition of adenosine 5'-monophosphate- and methacholine-induced bronchoconstriction in asthma by inhaled frusemide. *Eur Respir J* 1990; 3: 665–672.
5. Nichol GM, Alton EWFW, Nix A, Geddes DM, Chung KF, Barnes PJ. Effect of inhaled furosemide on metabisulfite and methacholine-induced bronchoconstriction and nasal potentials in asthmatic subjects. *Am Rev Respir Dis* 1990; 142: 576–580.
6. Rajakulasingam K, Polosa R, Church MK, Howarth PH, Holgate ST. Effect of inhaled frusemide on responses of airways to bradykinin and adenosine 5'-monophosphate in asthma. *Thorax* 1994; 49: 485–491.
7. Vaghi A, Robuschi M, Berni F, Bianco S. Effect of inhaled frusemide (F) on bronchial response to histamine (H) in asthmatics. *Eur Respir J* 1988; 1(Suppl. 2): 406S (Abstract).
8. Polosa R. Inhaled loop diuretics: how do we interpret their modulatory role in asthma? *Allergy* 1993; 48: 555–558.
9. Puschett JB. Sites and mechanisms of action of diuretics in the kidney. *J Clin Pharmacol* 1981; 21: 564–574.
10. O'Connor BJ, Chung KF, Chen-Wordsdell YM, Fuller RW, Barnes PJ. Effect of inhaled furosemide and bumetanide on adenosine 5'-monophosphate and sodium metabisulfite-induced bronchoconstriction in asthmatic subjects. *Am Rev Respir Dis* 1991; 143: 1329–1333.
11. Polosa R, Rajakulasingam K, Prosperini G, Church MK, Holgate ST. Relative potencies and time course of changes in adenosine 5'-monophosphate airway responsiveness with inhaled furosemide and bumetanide. *J Allergy Clin Immunol* 1993; 92: 288–297.
12. Mackay IG, Muir AL, Watson ML. Contribution of the prostaglandins to the systemic and renal vascular response to frusemide in man. *Br J Clin Pharmacol* 1984; 17: 513–519.
13. Miyanoshita A, Terada M, Endou H. Furosemide directly stimulates prostaglandin $E_2$ production in the thick ascending limb of Henlé's loop. *J Pharmacol Exp Ther* 1989; 251: 1155–1159.
14. Pavord ID, Knox AJ, Cole A, Tattersfield AE. Effect of frusemide on the release of prostaglandin $E_2$ by bovine tracheal mucosa. *Thorax* 1991: 46; 751 (Abstract).
15. Pavord ID, Wisniewski A, Mathur R, Wahedna I, Knox A, Tattersfield AE. Effect of inhaled prostaglandin $E_2$ on bronchial reactivity to sodium metabisulphite and methacholine in subjects with asthma. *Thorax* 1991; 46: 633–637.

16. Pasargiklian M, Bianco S, Allegra L, *et al.* Aspects of bronchial reactivity to prostaglandins and aspirin in asthmatic patients. *Respiration* 1977; 34: 79–91.
17. Pasargiklian M, Bianco S, Allegra L. Clinical, functional and pathogenetic aspects of bronchial reactivity to prostaglandins $F_{2\alpha}$, $E_1$ and $E_2$. *Adv Prostaglandin Thromboxane Res* 1976; 1: 461–475.
18. Fujimura M, Sakamoto S, Kamio Y. Effect of inhaled furosemide on bronchial responsiveness to methacholine. *N Engl J Med* 1990; 322: 935 (Letter).
19. Zamel N. Threshold of airway response to inhaled methacholine in healthy men and women. *J Appl Physiol Respirat Environ Exercise Physiol* 1984; 56: 129–132.
20. Stone RA, Barnes PJ, Chung KF. Effect of frusemide on cough responses to chloride-deficient solution in normal and mild asthmatic subjects. *Eur Respir J* 1993; 6: 862–867.
21. Folkerts G, Verheyen AKCP, Geuens GMA, Folkerts HF, Nijkamp FP. Virus-induced changes in airway responsiveness, morphology, and histamine levels in guinea-pigs. *Am Rev Respir Dis* 1993; 147: 1569–1577.
22. Polosa R, Lai CKW, Robinson C, Holgate ST. The influence of cyclo-oxygenase inhibition on the loss of bronchoconstrictor response to repeated bradykinin challenge in asthma. *Eur Respir J* 1990; 3: 914–921.
23. Pavord ID, Wisniewski A, Tattersfield AE. Inhaled frusemide and exercise-induced asthma: evidence of a role for inhibitory prostanoids. *Thorax* 1992; 47: 797–800.
24. O'Connor BJ, Barnes PJ, Chung KF. Inhibition of sodium metabisulphite induced bronchoconstriction by frusemide in asthma: role of cyclo-oxygenase products. *Thorax* 1994; 49: 307–311.
25. Holtzman MJ. Arachidonic acid metabolism: implications of biological chemistry for lung function and disease. Am Rev Respir Dis 1991; 143: 188–203.
26. Churchill L, Chilton FH, Resau JH, Bascom R, Hubbard WC, Proud D. Cyclo-oxygenase metabolism of endogenous arachidonic acid by cultured human tracheal epithelial cells. *Am Rev Respir Dis* 1989; 140: 449–454.
27. Lundergan CF, Fitzpatrick TM, Rose JC, Ramwell RN, Kot PA. Effect of cyclo-oxygenase inhibition on the pulmonary vasodilatator response to furosemide. *J Pharmacol Exp Ther* 1988; 246: 102–106.
28. Weber PC, Scherer B, Larsson C. Increase of free arachidonic acid by frusemide in man as the cause of prostaglandin and renin release. *Eur J Pharmacol* 1977; 41: 329–332.
29. Scherer B, Weber PC. Time-dependent changes in prostaglandin excretion in response to frusemide in man. *Clin Sci* 1979; 56: 77–81.
30. Goldberg M. The renal physiology of diuretics. *In*: Orloff J, Berliner RW, eds. Handbook of Physiology. Sect. 8. Renal Physiology. Washington DC, American Physiological Society, 1973; pp. 1003–1031.
31. Ito I, Suzuki H, Aizawa H, Hirose T, Hakoda H. Prejunctional inhibitory action of prostaglandin $E_2$ on excitatory neuro-effector transmission in the human bronchus. *Prostaglandins* 1990; 39: 639–655.
32. Bianco S, Robuschi M, Ceserani R, Gandolfio C. Effects of prostacyclin on aspecifically and specifically induced bronchoconstriction in asthmatic subjects. *Eur J Respir Dis* 1980; 106 (Suppl.): 81–87.
33. Hardy CC, Bradding P, Robinson C, Holgate ST. Bronchoconstrictor and antibronchoconstrictor properties of inhaled prostacyclin in asthma. *J Appl Physiol* 1988; 64: 1567–1574.
34. O'Byrne PM, Jones GL. The effect of indomethacin on exercise-induced bronchoconstriction and refractoriness after exercise. *Am Rev Respir Dis* 1986; 134: 69–72.
35. Mattoli S, Foresi A, Corbo GM, Valente S, Ciappi G. The effect of indomethacin on the refractory period occurring after the inhalation of ultrasonically nebulized distilled water. *J Allergy Clin Immunol* 1987; 79: 678–683.
36. Temple DM, Wei HE, Anderson SD. Inhibition by furosemide of inflammatory mediators from lung fragments. *N Engl J Med* 1990; 324: 131 (Letter).
37. Ventresca PG, Nichol GM, Barnes PJ, Chung KF. Inhaled furosemide inhibits cough induced by low chloride content solutions but not by capsaicin. *Am Rev Respir Dis* 1990; 142: 143–146.
38. Elwood W, Lotvall JO, Barnes PJ, Chung KF. Loop diuretics inhibit cholinergic and noncholinergic nerves in guinea-pig airways. *Am Rev Respir Dis* 1991; 143: 1340–1344.
39. Finnerty JP, Holgate ST. Evidence for the roles of histamine and prostaglandins as mediators in exercise-induced asthma: the inhibitory effect of terfenadine and flurbiprofen alone and in combination. *Eur Respir J* 1990; 3: 540–547.
40. Phillips GD, Holgate ST. The effect of oral terfenadine alone and in combination with flurbiprofen on the bronchoconstrictor responses to inhaled AMP in nonatopic asthma. *Am Rev Respir Dis* 1989; 139: 463–469.