Inhibition of sodium metabisulphite induced bronchoconstriction by frusemide in asthma: role of cyclooxygenase products

Brian J O'Connor, Peter J Barnes, K Fan Chung

Abstract

Background — Inhaled frusemide inhibits airway responses to sodium metabisulphite and other indirect bronchial challenges in asthma by undetermined mechanisms which may relate to its ability to stimulate prostaglandin release. Inhalation of sodium metabisulphite provokes indirect bronchoconstriction, possibly by activating sensory nerves. To investigate the role of cyclooxygenase products in the airway actions of frusemide and sodium metabisulphite, the effects of a potent cyclooxygenase inhibitor, flurbiprofen, alone and in combination with frusemide were investigated against airway responsiveness to sodium metabisulphite.

Methods — In a double blind double placebo controlled study, 12 mild asthmatic subjects attended on four occasions to undergo three inhalation challenges with sodium metabisulphite. A baseline challenge was performed one hour before oral intake of flurbiprofen 200 mg or matched placebo, and two hours before inhalation of frusemide 40 mg or matched placebo. A second challenge was performed immediately after inhalation of frusemide (two hours after flurbiprofen) with a further challenge three hours later. The log concentration provoking a 20% fall in FEV1 (log PC20) was used to assess airway responsiveness to sodium metabisulphite.

Results — Frusemide caused an immediate 1/9 doubling dose protection and a lesser 0/7 doubling dose protection at three hours. This protection was enhanced by flurbiprofen at both time points to 2/7 (early) and 1/9 (late) doubling doses. In addition, flurbiprofen alone significantly reduced airway responsiveness to sodium metabisulphite by 1/1 doubling doses at both two and five hours.

Conclusions — The generation of broncho-protective prostaglandins is unlikely to underlie the inhibitory action of frusemide against airway responsiveness to sodium metabisulphite. Endogenous contractile prostaglandins within the airways may be involved in the bronchoconstricor response to sodium metabisulphite.

(Thorax 1994;49:307–311)
the protective effect of frusemide persisted for up to three hours.

Methods
SUBJECTS
Twelve non-smoking mild asthmatic subjects (table 1) gave informed consent to participate in the study which was approved by the Royal Brompton and National Heart Hospitals ethics committee. All were atopic as defined by a positive cutaneous response to intradermal challenge with common airborne allergens (Dermatophagoides pteronyssinus, mixed grass pollen, cat fur, and dog hair). None had suffered an exacerbation of wheeze nor a respiratory infection in the preceding six weeks. All were controlled on inhaled β agonist therapy alone and had a baseline forced expiratory volume in one second (FEV₁) in excess of 80% of their predicted value. All had taken aspirin and other non-steroidal anti-inflammatory drugs without adverse reaction on a number of occasions but not within four weeks before participating in the study.

STUDY DESIGN
This was a balanced randomised double blind double placebo crossover study in which each subject attended on four different days, each separated by at least one week. Inhaled β agonists and caffeinated beverages were withheld for at least eight hours before each visit. On each study day subjects underwent a baseline inhalation challenge with sodium metabisulphite. One hour later subjects took flurbiprofen 200 mg or matched placebo in a single oral dose. After an interval of two hours each subject then inhaled either frusemide 40 mg or placebo by continuous nebulisation for 15 minutes immediately before a second sodium metabisulphite challenge. A third sodium metabisulphite challenge was performed three hours after frusemide - that is, five hours after flurbiprofen. Thus, on the four visits subjects were challenged with sodium metabisulphite at three time points, once before and twice after treatment with either (1) placebos alone; (2) oral flurbiprofen 200 mg; (3) inhaled frusemide 40 mg; or (4) a combination of flurbiprofen and frusemide.

Table 1: Characteristics of subjects taking part in study

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age (years)</th>
<th>Gender</th>
<th>FEV₁ (%) predicted</th>
<th>Baseline PC₂₀</th>
<th>MB5 (mg/ml)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>M</td>
<td>108</td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>F</td>
<td>104</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>F</td>
<td>83</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>M</td>
<td>102</td>
<td>18.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>F</td>
<td>93</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>M</td>
<td>88</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>M</td>
<td>81</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>M</td>
<td>107</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>M</td>
<td>91</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>31</td>
<td>F</td>
<td>82</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>27</td>
<td>F</td>
<td>90</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>32</td>
<td>F</td>
<td>84</td>
<td>2.1</td>
<td></td>
</tr>
</tbody>
</table>

FEV₁ = forced expiratory volume in one second; MB5 = sodium metabisulphite.

Materials and Drug Delivery
On each study day fresh solutions of sodium metabisulphite (Sigma, UK) were made up to produce a range of concentrations in 0.9% saline of 0.3–80 mg/ml. Each solution was administered from a nebuliser attached to a breath activated dosimeter (Mefar, Brescia, Italy). The nebuliser delivered particles with an aerodynamic mass median diameter of 3.5–4 μm at an output of 10 μl/breath. The dosimeter was set to nebulise for one second with a pause time of eight seconds at a pressure of 22 psi.

Flurbiprofen (Boots, Nottingham, UK) or matched placebo was administered as four 50 mg capsules. Frusemide (10 mg/ml in distilled water containing sodium hydroxide 1.2 mg/ml, pH 5.3, osmolarity 209 mosmol) (Antigen, Roscrea, Ireland) or matched placebo (pH 5.6, osmolarity 225 mosmol) was delivered by continuous nebulisation from an Acorn Jet nebuliser (Medic-aid, Sussex, UK) with a calibrated output of 0.25 ml/min at a driving pressure of 61/min. The nebuliser delivered an aerosol with aerodynamic diameter of 3.5–μm at this flow rate as measured by a laser particle sizer (Model 2600d, Malvern, Worcestershire, UK).

Bronchial Provocation and Measurement of Pulmonary Function
Pulmonary function was measured as FEV₁ with a dry wedge spirometer (Vitalograph, Buckinghamshire, UK). A standard challenge protocol was used for all provocation tests. On arrival in the laboratory each subject rested quietly for 15 minutes before three measurements of FEV₁, taken at one minute intervals. Subjects then inhaled five breaths of saline control by inspirating slowly from functional residual capacity to total lung capacity over three seconds and then breath holding for five seconds. FEV₁ was measured two minutes after inhalation of saline. Unless a fall in FEV₁ of greater than 10% was seen, subjects inhaled five breaths of doubling concentrations of sodium metabisulphite starting at 0.3 mg/ml until a greater than 20% fall in FEV₁ from the post saline value was achieved. A log dose-response curve was constructed and the provocative concentration causing a 20% fall in FEV₁ was calculated by linear interpolation and expressed in logarithmic terms (log PC₂₀). Challenges were performed at the same time of the day for each individual.

Data Analysis
Log PC₂₀ values for sodium metabisulphite before and at both time points after active and placebo treatments were compared using analysis of variance for multiple comparisons and the change assessed by the least significant difference test. The protective effect of each treatment on responses to sodium metabisulphite at both time points was calculated by measuring the change in log PC₂₀ from the baseline after all active and placebo treatments.
and expressed in terms of doubling doses using the formula:

\[(\text{post} - \text{pre}) \log \text{PC}_{20} \text{active} - (\text{post} - \text{pre}) \log \text{PC}_{20} \text{placebo}/\log 2\]

Baseline spirometric results before and at both time points after each treatment were also compared using analysis of variance for multiple comparisons.

Results are expressed as mean (SE). A p value of < 0.05 was regarded as significant.

### Results

Baseline spirometric values were unchanged throughout all treatment periods. Neither flurbiprofen nor frusemide, alone or in combination, caused any significant bronchodilatation (table 2). No side effects were reported by any subject after any of the treatment periods.

Baseline log \( \text{PC}_{20} \) sodium metabisulphite was similar on all four study days (figure). On the placebo limb, log \( \text{PC}_{20} \) responses were unchanged throughout the day (0.84 (0.10) before, and 0.83 (0.11) and 0.84 (0.09) at both time points after placebo) (figure).

### Discussion

This study confirms that inhaled frusemide inhibits sodium metabisulphite induced bronchoconstriction and that the duration of this inhibition can last for up to three hours. This protection is significantly enhanced by a single oral dose of flurbiprofen, increasing the early and late effects of frusemide from 1.9 to 2.7 and from 0.7 to 1.9 doubling doses respectively. Furthermore, flurbiprofen alone reduced the constrictor response to sodium metabisulphite by 1.1 doubling doses at both two and five hours after treatment.

Our results show that the release of prostaglandins such as PGE, is unlikely to mediate the protective effect of frusemide against sodium metabisulphite induced bronchoconstriction. Flurbiprofen did not inhibit but, rather, enhanced the actions of frusemide. The interpretation of this enhancement is complicated by the observation that flurbiprofen alone significantly protected against sodium metabisulphite challenge. This protection of 1.1 doubling doses at both time points, when added to the 1.9 (early) and 0.7 (late)

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**Table 2** Mean (SE) forced expiratory volume in one second (FEV₁, litres) before (pre) and at both time points, early (post 1) and late (post 2), after treatment

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post 1</th>
<th>Post 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>3.28 (0.30)</td>
<td>3.23 (0.29)</td>
<td>3.28 (0.29)</td>
</tr>
<tr>
<td>Frusemide</td>
<td>3.34 (0.28)</td>
<td>3.28 (0.29)</td>
<td>3.40 (0.29)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>3.25 (0.27)</td>
<td>3.19 (0.28)</td>
<td>3.24 (0.30)</td>
</tr>
<tr>
<td>Frusemide + flurbiprofen</td>
<td>3.25 (0.29)</td>
<td>3.21 (0.27)</td>
<td>3.18 (0.29)</td>
</tr>
</tbody>
</table>

FRUSEMIDE VS SODIUM METABISULPHITE

Inhalation of frusemide alone significantly reduced responsiveness to sodium metabisulphite at both time points (log \( \text{PC}_{20} \) 0.88 (0.09) before, 1.46 (0.13) immediately after, and 1.09 (0.12) three hours after treatment) (figure). Thus frusemide displaced the sodium metabisulphite dose-response curve to the right by 1.9 doubling doses immediately (p < 0.01) and by 0.7 doubling doses at three hours (p < 0.05).

FLURBIPROFEN VS SODIUM METABISULPHITE

Flurbiprofen alone significantly attenuated the constrictor response to sodium metabisulphite (log \( \text{PC}_{20} \) 0.85 (0.11) before, 1.17 (0.15) at two hours, and 1.18 (0.15) at five hours after treatment) (figure). Flurbiprofen therefore displaced the dose-response curve of sodium metabisulphite to the right by 1.1 doubling doses at both time points compared with placebo (p < 0.01).

FLURBIPROFEN AND FRUSEMIDE IN COMBINATION VS SODIUM METABISULPHITE CHALLENGE

Combined treatment with flurbiprofen and frusemide had a greater effect on log \( \text{PC}_{20} \) sodium metabisulphite than either treatment alone (0.85 (0.11) before treatment, 1.67 (0.16) two hours after flurbiprofen and five minutes after frusemide, 1.38 (0.16) five hours after flurbiprofen and three hours after frusemide) (figure). The combination therapy therefore displaced the sodium metabisulphite dose-response curve to the right by 2.7 doubling doses at the early time point (p < 0.001) and by 1.9 doubling doses at the later time point (p < 0.001) when compared with placebo. This shift in the dose-response curve was significantly greater than after either agent alone (p < 0.01).
doubling dose protection of frusemide alone, is
equivalent to the protection of both agents in
combination. The protection observed with
the combination of flurbiprofen and frusemide
is therefore likely to be additive, implying that
the mechanism of protection of frusemide
against sodium metabisulphite is unaffected by
cyclooxygenase inhibition.

The lack of significant involvement of
bronchoprotective prostaglandins in the air-
way actions of frusemide is supported by the
recent observation that aerosolised lysine-
 aspirin did not influence the inhibitory effect of
frusemide against exercise induced asthma.16 In a recent study of similar design,
however, another cyclooxygenase inhibitor
(indomethacin, 50 mg three times daily for
three days) reduced the protective effect of
frusemide against exercise induced asthma in
the absence of an effect of indomethacin alone,
implying that during exercise frusemide may
generate bronchoprotective prostaglandins
such as PGE2 and PG12, within the airways.17 A
differential effect of these prostaglandins on
different bronchial challenges cannot explain
the difference in the two studies because both
PGE2 and PG12 protect against bronchocon-
striction induced by sodium metabisulphite and
exercise.911 The disparity between these
data and the results of our recent study may be
a result of the differences in the properties of
flurbiprofen and indomethacin. Flurbiprofen
inhibits the immediate bronchoconstrictor re-
sponse to allergen,48 exercise,19 and adenos-
ine,20 whereas indomethacin has little effect on
these challenges.172122 Although we cannot ac-
count for the contrasting effects of these two
agents on asthmatic airways, it is possible that
flurbiprofen is a more potent and specific inhib-
itor of airway cyclooxygenase than indometh-
acin. To answer this question definitively it
would be necessary to perform a study com-
paring the effect of a single equipotent dose of
each agent against sodium metabisulphite
induced bronchoconstriction.

Our findings suggest that frusemide is un-
likely to generate bronchoprotective prostas-
glandins. However, frusemide causes therenal
release, not only of PGE2,27 but also of contrac-
tile PGF2α,28 It is possible the inhalation of
frusemide may generate prostaglandins with
opposing effects on airway smooth muscle, but
we feel that this is unlikely. Both PGE2 and
PGF2α are tussive agents;24 if frusemide was
generating significant airway levels of either
prostaglandin it should have provoked cough,
but there have been no such reports.4546 Indeed,
frusemide inhibits responses to different cough
challenges29-30 providing further circumstantial
evidence against a role for prostaglandins
mediating its airway actions.

Flurbiprofen alone inhibited the broncho-
constrictor response to sodium metabisul-
phite. This effect is unlikely to result from a
reduction in non-specific airway responsive-
ness as previous studies with this drug have
failed to show an effect on histamine or meta-
choline induced bronchoconstriction.1820 The
reduction in constrictor responses to other
indirect challenges such as adenosine, allergen,
and exercise18-20 has been attributed to the
ability of flurbiprofen to inhibit synthesis and
release of airway derived contractile prosta-
glandins. The inhibitory action of flurbiprofen
observed in this study suggests that sodium
metabisulphite may release contractile pro-
tactile prostaglandins from airway cells or,
alternatively, that prostaglandins present in
asthmatic airways may potentiate the constrict-
or response to sodium metabisulphite. Sodium
metabisulphite does not appear to
stimulate the release of histamine14 but it may
activate sensory nerves.1115 Prostaglandins are
neuromodulators, stimulate airway sensory
nerves,27 and augment neurotransmission in
efferent airway nerves.28 These observations
suggest that endogenous prostaglandins may
potentiate the response of asthmatic airways
to inhaled sodium metabisulphite and that
the effects of sodium metabisulphite may
partly result from the release of contractile
prostaglandins.

The immediate 1-9 doubling dose protection
afforded by frusemide against sodium metabi-
sulphite is similar to that seen in our previous
study.1 We have shown previously both that
frusemide provides a small but significant 0-7 doubling
dose protection against sodium metabisulphite
at three hours. This is the first study to show
such sustained inhibition of bronchoconstric-
tion. Our current data and the recent observa-
tion of a reduction in the cough response to
chloride-free solutions for up to and including
four hours29 therefore support a prolonged
airway action of frusemide.

In summary, we have shown that the protec-
tive effect of frusemide against the indirect
bronchoconstrictor stimulus sodium metabi-
sulphite is sustained for up to three hours and
is unlikely to involve release of bronchoprotective
prostaglandins. On the other hand, the
bronchoconstrictor response to sodium meta-
sulphite appears to be partially mediated through
the actions of cyclooxygenase products present in
asthmatic airways. The pre-
cise role of contractile prostaglandins in the
 genesis of sodium metabisulphite induced
bronchoconstriction requires further elucidation.

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icals for kindly donating flurbiprofen and matched placebos.

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Effect of flurbiprofen and frusemide on airway responsiveness to sodium metabisulphite

Inhibition of sodium metabisulphite induced bronchoconstriction by frusemide in asthma: role of cyclooxygenase products.
B J O'Connor, P J Barnes and K F Chung

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