Comparison of three inhaled non-steroidal anti-inflammatory drugs on the airway response to sodium metabisulphite and adenosine 5'-monophosphate challenge in asthma

Millie Wang, Antoni Wisniewski, Ian Pavord, Alan Knox, Anne Tattersfield

Abstract

Background – Non-steroidal anti-inflammatory drugs (NSAIDs) are used to assess the role of prostaglandins in asthma but their effects on bronchoconstrictor challenges have been inconsistent. The effects of three nebulised non-steroidal anti-inflammatory drugs on the airway response to inhaled sodium metabisulphite (MBS) and adenosine 5'-monophosphate (AMP) were compared in the same asthmatic subjects to see whether contractile prostaglandins were involved in MBS or AMP induced bronchoconstriction. A possible protective effect of the osmolarity or pH of the inhaled solutions was also assessed.

Methods – Two double blind placebo controlled studies were carried out. In study 1, 15 non-aspirin sensitive patients with mild asthma attended on four occasions and inhaled 5 ml of lysine aspirin (L-aspirin) 900 mg, indomethacin 50 mg, sodium salicylate 800 mg, or saline 20 minutes before an inhaled MBS challenge. On four further occasions 14 of the patients inhaled the same solutions followed by an inhaled AMP challenge. In study 2, 10 of the patients attended on four additional occasions and inhaled 5 ml of 0.9%, 3%, 10%, or 9.5% saline with indomethacin 50 mg 20 minutes before an inhaled MBS challenge.

Results – In study 1 inhaled lysine aspirin had a similar effect on MBS and AMP induced bronchoconstriction, increasing the provocative dose causing a 20% fall in FEV₁ (PD_{20}) by 1.29 (95% CI 0.54 to 2.03) and 1.23 (95% CI 0.53 to 1.93) doubling doses, respectively. Indomethacin increased the MBS PD_{20} and AMP PD_{20} by 0.64 (95% CI 0.1 to 1.38) and 0.99 (95% CI 0.29 to 1.69) doubling doses, respectively. Sodium salicylate had no significant effect on either challenge. The two solutions causing most inhibition were the most acidic and the most alkaline. In study 2 inhaled 9.5% saline with indomethacin (osmolarity 3005 mOsm/kg) increased the MBS PD_{20} by 1.1 doubling doses (95% CI 0.2 to 2.0) compared with only 0.09 (95% CI −0.83 to 1.0) and 0.04 (95% CI −0.88 to 0.95) doubling doses with 3% saline (918 mOsm/kg) and 10% saline (2994 mOsm/kg), respectively.

Conclusions – Inhaled L-aspirin and indomethacin have broadly similar protective effects against MBS and AMP induced bronchoconstriction in the doses given, although the effect of indomethacin on MBS was not quite statistically significant. The osmolarity and pH of the solutions did not appear to be important determinants of the response. The effect of L-aspirin and indomethacin is likely to be the result of cyclooxygenase inhibition reducing the production of contractile prostaglandins during MBS and AMP challenge.

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Keywords: asthma, non-steroidal anti-inflammatory drugs, prostaglandin.
indomethacin protect against ultrasonically nebulised distilled water induced bronchoconstriction, suggesting that bronchoconstrictor tone in these circumstances is prostaglandin dependent. Inhibitory prostaglandins such as PGE_{2} appear to have a role in the development of refractoriness following indirectly acting challenges since this is inhibited by NSAIDs, and there is some evidence to suggest that they mediate the effects of frusemide although this has been less consistent. The protective effect of frusemide on bronchoconstriction following exercise was attenuated by oral indomethacin in one study but augmented by inhaled lysine aspirin and oral flurbiprofen in others. Thus, there is evidence that both constrictor and protective prostaglandins play a part in determining airway calibre under certain circumstances, although there are many unexplained inconsistencies in the literature.

One problem when comparing studies on the effects of NSAIDs in asthma is the considerable variation in the drugs given, doses, routes of administration, osmolarity and pH of the inhaled solutions, and the bronchial challenge tests used to assess the response. NSAIDs are a non-homogeneous group of chemical compounds with varying effects on the two cyclooxygenase isoenzymes currently identified, COX-1 and COX-2. Discrepancies in the literature may therefore be due to differences in the drugs, the methodologies used, or to differences in osmolarity or pH of the inhaled solutions.

The bronchoconstriction caused by sodium metabisulphite is thought to act via release of sulphur dioxide (SO_{2}) and activation of neural pathways. In contrast, the bronchoconstriction caused by inhalation of adenosine and its related nucleotide, adenosine 5'-monophosphate (AMP), appears to be largely due to histamine release from primed airway mast cells, selective H_{1}-receptor antagonists such as terfenadine inhibit the response to AMP in patients with asthma but not the response to sodium metabisulphite. The effect of inhaled NSAIDs on the airway response to inhaled MBS and AMP has not been studied. We have therefore compared the protective effect of three nebulised NSAIDs against inhaled MBS and AMP induced bronchoconstriction in asthmatic subjects who were not sensitive to aspirin to see whether contractile prostaglandins were involved in these challenges. We also assessed whether the osmolarity or pH of the inhaled solutions were related to any protective effect.

**Methods**

**SUBJECTS**

Fifteen non-smoking subjects (one woman) aged 21–51 years with mild asthma and no history of aspirin induced asthma were studied. Twelve were atopic (two or more positive skin prick tests to common allergens). All had asthma that was well controlled, a forced expiratory volume in one second (FEV_{1}) greater than 70% predicted, and no evidence of bronchoconstriction in response to inhaled lysine aspirin in the laboratory (see below). All were taking an inhaled β_{2} agonist as required, six were taking inhaled beclomethasone (100–200 μg per day), and one took oral loratadine as required for rhinitis. Inhaled β agonists were withheld for at least 10 hours and oral loratadine for at least 48 hours before each challenge. Subjects were asked to use only paracetamol for pain relief if required during the study period. The study was approved by the City Hospital ethics committee and all subjects gave written informed consent.

**MATERIALS**

The drugs and chemicals used in the study included lysine aspirin (Laboratori Synthelabo, Paris, France), indomethacin meglumine (Liometacen, Chiesi Farmaceutici, Parma, Italy), sodium salicylate (E Merck, UK), saline = 0.9% (w/v) sodium chloride (Steripak Ltd, UK), 30% sodium chloride (Martindale Pharmaceuticals Ltd, Essex, UK), sodium metabisulphite (MBS) (Thornton & Bissell Ltd, UK), and adenosine 5'-monophosphate (AMP) (Sigma, Poole, Dorset, UK).

Forced expiratory volume in one second (FEV_{1}) was measured with a dry wedge spirometer (Vitalograph, Buckingham, UK) and the higher of two successive readings within 100 ml was recorded.

Sodium metabisulphite and adenosine 5'-monophosphate were dissolved freshly in saline to produce a doubling concentration range of 0.6–160 mg/ml for sodium metabisulphite and 3.12–200 mg/ml for adenosine 5'-monophosphate. Solutions were delivered by a nebuliser attached to a breath actuated dosimeter (Mefar, Brescia, Italy) at a nebulisation time of one second, pause time six seconds, pressure 22 lb/m (152 kPa), output 10.3 μl and 12.8 μl per puff for MBS and AMP, respectively. Normal saline was inhaled initially with the subjects inspirng slowly through the mouthpiece from functional residual capacity to total lung capacity over three seconds after the nebuliser was triggered and then breath holding for three seconds. FEV_{1} was measured after two minutes. If the response to saline was less than a 10% fall from baseline FEV_{1}, subjects inhaled doubling doses of MBS or AMP until a 20% or greater fall in FEV_{1} was recorded or the maximum dose of MBS (135 μmol) or AMP (118 μmol) had been given.

**PROTOCOL**

**Screening study**

An initial inhaled aspirin challenge was performed using a disposable nebuliser (Devilbiss, Somerset) according to the method of Phillips et al. 1800 mg lysine aspirin (L-aspirin) powder (equivalent to aspirin 1000 mg) was made up freshly each day in 5 ml saline to produce a 360 mg/ml L-aspirin solution. This solution was diluted further in saline to produce doubling concentrations from 5.6 to 360 mg/ml. Three ml of each solution was placed in the nebuliser which was driven by compressed air at 8 l/min (nebuliser output 0.41 ml/min). Subjects wore a nose clip and inhaled five breaths of normal saline, inspirin-
Effect of NSAIDs on airway responses in asthma

Effect of NSAIDs on slowing from functional residual capacity to total lung capacity followed by a three second breath hold. FEV₁ was measured five minutes later and followed by 10 breaths of L-aspirin at 15 minute intervals in doubling concentrations starting at 5.6 mg/ml. FEV₁ was measured five, 10, and 15 minutes after each concentration. The test was discontinued if the FEV₁ fell more than 10% after saline or L-aspirin compared with the value after saline.

Subjects only entered the two main studies if FEV₁ fell by less than 10% with the highest dose of L-aspirin (360 mg/ml). Both studies were double blind and placebo controlled.

Study 1: Effect of three NSAIDs on the airway response to MBS and AMP

Subjects attended for eight visits at the same time of day, each visit separated by at least 72 hours. After resting for 15 minutes they inhaled one of the following drugs dissolved in 5 ml saline and made up freshly by a second investigator: 900 mg L-aspirin (equivalent to 500 mg aspirin), 77.2 mg indomethacin meglumine (equivalent to 50 mg indomethacin), 800 mg sodium salicylate, or placebo (saline). The solutions were administered using a jet nebuliser (Unineb, Unimed (UK) Ltd, Dorset, UK) and mask driven by compressed air at 8 l/min and run by regular breathing until the nebuliser mist was no longer visible (output 0.36 ml/min). The same nebuliser was used throughout the study. The pH and osmolality of the solutions were measured before administration by a pH meter (MB2 Radiometer A/S, Copenhagen, Denmark) and an osmometer (Osmometry 030, Gonotec GmbH, Berlin, Germany). FEV₁ was recorded before and at two, five, 10 and 20 minutes after treatment. At 20 minutes patients underwent an inhaled MBS challenge during the first four visits and an inhaled AMP challenge on the second four visits.

Study 2: Osmolarity study

Ten patients from the first study attended on four further occasions. 30% sodium chloride was diluted in sterile water to produce concentrations of 3%, 10%, and 9.5% sodium chloride. Subjects inhaled 5 ml of each of the following: 0.9% saline (292 mOsm/kg), 3% saline (918 mOsm/kg), 10% saline (2994 mOsm/kg), or 9.5% saline with indomethacin 50 mg (3005 mOsm/kg) 20 minutes before an inhaled MBS challenge as described above.

Based on our previous data, the study had 95% power to detect a difference of 1.4 doubling doses at the 5% level of significance.

DATA ANALYSIS

Two way analysis of variance (ANOVA) was used to compare baseline values of FEV₁ and the change in FEV₁ at two, five, 10, and 20 minutes after each drug. The dose of bronchoconstrictor agent causing a 20% fall in FEV₁ from baseline (PD₂₀, FEV₁) was calculated by linear interpolation of the log dose/response curve. If the fall in FEV₁ was less than 20% after the maximum dose of 135 μmol MBS or 118 μmol AMP the curve was extrapolated by one doubling dose to provide an estimated PD₂₀. If the estimate was greater than the next doubling dose this value (270 or 235 μmol) was used as a censored value. This occurred on 11 of 60 occasions after MBS challenge and on 14 of 56 occasions after AMP challenge. The distribution of censored values after saline, L-aspirin, sodium salicylate, and indomethacin were two, three, two, and four occasions, respectively, for MBS and one, five, four, and four occasions after AMP (for MBS one subject had censored values after all four solutions and one after all but sodium salicylate; for AMP one subject had censored values after all four solutions and two after all three NSAIDs). A censored value was used on five of 40 occasions of the PD₂₀ MBS in study 2 (one, three, and one occasion after 0.9% saline, indomethacin with 9.5% saline, and 3% saline, respectively; one subject had a censored value after all three solutions).

PD₂₀ values were log transformed and are presented as geometric mean values. The log values were compared using ANOVA. The mean difference in PD₂₀ values relative to saline are expressed as doubling doses with 95% confidence intervals (CI). The difference in doubling doses was calculated as: [log MBS or AMP PD₂₀ (drug)] – [log MBS or AMP PD₂₀ (saline)]/log 2.

Results

Study 1: Effect of three NSAIDs on PD₂₀ MBS and PD₂₀ AMP

The drugs were well tolerated apart from sodium salicylate which caused short lived cough in all subjects and prevented two subjects from providing an FEV₁ measurement two minutes after treatment. Only 14 of the 15 subjects were available for the AMP study. The pH of the solutions in the nebuliser ranged from 5.18 (L-aspirin) to 7.92 (indomethacin) and the osmolality from 295 (saline) to 2100 mOsm/kg (sodium salicylate) (table 1).

Mean baseline FEV₁ did not differ significantly between the four MBS or four AMP

<table>
<thead>
<tr>
<th>Study</th>
<th>pH</th>
<th>Osmolarity (mOsm/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>0.9% saline</td>
<td>5.80</td>
</tr>
<tr>
<td></td>
<td>Lysine aspirin</td>
<td>5.18</td>
</tr>
<tr>
<td></td>
<td>Sodium salicylate</td>
<td>6.39</td>
</tr>
<tr>
<td></td>
<td>Indomethacin</td>
<td>7.92</td>
</tr>
<tr>
<td>Study 2</td>
<td>0.9% saline</td>
<td>5.80</td>
</tr>
<tr>
<td></td>
<td>3% saline</td>
<td>6.50</td>
</tr>
<tr>
<td></td>
<td>10% saline</td>
<td>6.80</td>
</tr>
<tr>
<td></td>
<td>9.5% saline + indomethacin</td>
<td>8.20</td>
</tr>
</tbody>
</table>
There were no returned L-aspirin. When the two subjects with three and four censored values were excluded the PD<sub>20</sub> values increased to 1.49, 0.84, and 0.79 doubling doses after L-aspirin, indomethacin, and sodium salicylate, respectively, the increase for indomethacin being then statistically significant (p = 0.05) and that for sodium salicylate being almost significant (p < 0.1). The changes to the PD<sub>20</sub> AMP values after the subjects with censored values were excluded were minor and did not alter the statistical significance of the findings.

Study 2: Osmolarity study

All four solutions were well tolerated. The pH ranged from 5.8 (0.9% saline) to 8.2 (indomethacin with 9.5% saline) and the osmolarity from 292 (0.9% saline) to 3005 mOsm/kg (9.5% saline with indomethacin) (table 1). The mean baseline FEV<sub>1</sub> did not differ significantly between the four study days.

There was a small increase from baseline in FEV<sub>1</sub> following inhalation of normal saline (0.4%) and a small decrease following the other three solutions (range 1.8–5.7%) causing a difference between the four solutions which was statistically significant at two minutes after inhalation (p = 0.05) but not at five, 10, or 20 minutes.

There was a significant difference in MBS PD<sub>20</sub> between the three hyperosmolar solutions (p < 0.05). When compared with the PD<sub>20</sub> values following saline, PD<sub>20</sub> MBS increased by 1.29 doubling doses with L-aspirin (95% CI 0.54 to 2.03), by 0.64 doubling doses with indomethacin (95% CI −0.1 to 1.38), and by 0.63 doubling doses with sodium salicylate (95% CI −0.11 to 1.38). There was no significant difference in PD<sub>20</sub> MBS values between the three drugs.

PD<sub>20</sub> AMP was significantly higher after L-aspirin and indomethacin (fig 1). When compared with PD<sub>20</sub> values following saline, PD<sub>20</sub> AMP increased by 1.23 doubling doses with L-aspirin (95% CI 0.53 to 1.93), by 0.99 doubling doses with indomethacin (95% CI 0.29 to 1.69), and by 0.31 doubling doses with sodium salicylate (95% CI −0.39 to 1.01). The difference in PD<sub>20</sub> AMP between the three drugs was significant (p < 0.05).

When the two subjects with three and four censored values were excluded the PD<sub>20</sub> AMP values increased to 1.49, 0.84, and 0.79 doubling doses after L-aspirin, indomethacin, and sodium salicylate, respectively, the increase for indomethacin being then statistically significant (p = 0.05) and that for sodium salicylate being almost significant (p < 0.1). The changes to the PD<sub>20</sub> AMP values after the subjects with censored values were excluded were minor and did not alter the statistical significance of the findings.

Discussion

We have compared the effects of L-aspirin, indomethacin, and sodium salicylate against two bronchoconstrictor challenges with different modes of action in patients with mild asthma who were not aspirin sensitive. Most patients with asthma show bronchial hyper-responsiveness to a large number of stimuli. These may act directly on airways smooth muscle or indirectly by stimulating neural
reflexes (for example, metabisulphite) or by causing inflammatory cell mediator release (for example, exercise, AMP).

Three NSAIDs (L-aspirin, indomethacin, and sodium salicylate) were given by the same route to the same patients. None caused a change in FEV₁ compared with saline in study 1, confirming the absence of aspirin sensitive asthma in these patients. Pretreatment with inhaled L-aspirin (900 mg) protected against bronchoconstriction induced by MBS and AMP when compared with saline, causing a 1.3 and 1.2 doubling dose increase in PD₂₀ values, respectively. Inhaled indomethacin (50 mg) shifted the dose-response curves of MBS and AMP challenge to the right by 0.6 and 1.0 doubling doses, respectively, although only the change with the 1.0 doubling dose was statistically significant. The small changes in PD₂₀ MBS and AMP after inhaled sodium salicylate were not significant (0.6 and 0.3 doubling doses, respectively). The protective effect of inhaled indomethacin against MBS challenge may have been underestimated as four of the PD₂₀ values after indomethacin were censored. When results from the two protecitives with three or more censored values were excluded from the analysis the increase in PD₂₀ MBS (0.84 doubling doses) was statistically significant.

Sodium salicylate is a weak cyclooxygenase inhibitor compared with both aspirin and indomethacin,²¹⁻²³ and inhaled L-aspirin and indomethacin were active in our study whereas sodium salicylate was not. We have considered that the protection is due to cyclooxygenase inhibition.

In this study L-aspirin 900 mg and indomethacin 50 mg had broadly similar effects on the response to MBS and AMP – two bronchoconstrictor agents with different mechanisms of action. The same doses of L-aspirin and indomethacin caused a similar increase in ultrasonically nebulized water PD₂₀ (2.1 and 1.6 doubling doses) compared with placebo in patients with asthma studied by Bianco.²⁴ If the two drugs are causing protection through COX inhibition, both MBS and AMP induced bronchoconstriction must be dependent on release of contractile prostanoids (or potentiated by prostanoids in the airways) to a roughly similar extent. Sodium metabisulphite is thought to activate sensory nerves.²⁵ PGD₂ can enhance cholinergic neurotransmission.²⁷

It has been shown that inhaled ipratropium bromide has a protective effect on the airway response to inhaled PGD₂,²⁸ which suggests that PGD₂ augments the parasympathetic contractile response prejunctionally and this is likely to involve the accelerated release of acetylcholine at the neuromuscular junction.²⁷ It follows that PGD₂ could potentiate the neural response to sodium metabisulphite. Inhibition of COX would reduce PGD₂ production and thereby reduce the response to MBS. It has been shown that exogenous histamine induces release of PGF₂α in the airways in vitro.²⁹ It is feasible that histamine released from mast cells may produce some of its bronchoconstrictor effect by releasing contractile cyclooxygenase products. Thus, COX inhibitors could attenuate AMP induced bronchoconstriction by this mechanism.

If the effect of aspirin and indomethacin is due to COX inhibition, their relative effects on MBS and AMP challenges should reflect their relative potencies as COX inhibitors. Cyclooxygenase exists as two isozymes, COX-1 and COX-2, COX-1 being a constitutive enzyme involved in cellular “housekeeping” functions, whereas COX-2 is an inducible enzyme which is increased in the presence of inflammation, and is responsible for the production of proinflammatory prostanoids. When the relative potency of aspirin and indomethacin in inhibiting cyclooxygenase has been studied in broken cell, purified enzymes and intact cell preparations both drugs have been more potent inhibitors of COX-1 than COX-2, although the ratio of COX-1 to COX-2 inhibition has differed depending on the preparation studied.³⁰ It is difficult therefore to predict the relative potency of aspirin and indomethacin in inhbiting the two COX enzymes in the airways other than to say that both drugs are likely to be more selective for COX-1.

If the potent effect of NSAIDs in asthma is due to COX-2 inhibition, larger doses would be needed and this is more likely to be achieved by inhalation. High doses of inhaled NSAIDs have caused consistent inhibition of bronchoconstrictor responses as did oral indomethacin and flurbiprofen when given before adenosine induced bronchoconstriction.³¹ ³² Oral indomethacin (50 mg three times daily) for three days did not protect against MBS induced bronchoconstriction,³³ however, in contrast to the findings with inhaled indomethacin. The lower concentration of indomethacin in the airways following oral administration may not have been in the millimolar range needed to inhibit COX-2.³⁴ Induction of COX-2 has not yet been demonstrated in mast cells from asthmatic patients, however.

We also considered whether production of 15-HETE (15-hydroxy eicosatetraenoic acid) might contribute to the protective effect of aspirin since it increases in response to aspirin in cultured ovine tracheal epithelial cells,³⁵ and can inhibit the 5-lipoxygenase pathway in neutrophils and T lymphocytes.³⁶ ³⁷ Since it is not increased with indomethacin,³⁸ it is unlikely to play an important part in the protection seen in our study.

In study 2 all three hyperosmolar solutions caused a transient decrease in FEV₁, from baseline compared with 0.9% saline which returned to placebo values after two minutes. Inhaled indomethacin (50 mg) with 9.5% saline protected against MBS challenge and the magnitude of this effect (1.1 doubling doses) was similar to that seen in study 1 with the same dose of indomethacin in 0.9% saline in the same subjects (0.9 doubling doses). The 3% and 10% saline solutions had no effect. The osmolarity of the solutions does not therefore appear to be an important determinant of the protective effect. Hyperosmolar saline induced bronchoconstriction has been shown to cause refractoriness,³⁹ but whether cross refractoriness occurs to affect
other bronchial provocation challenges has not been studied. The lack of effect of hyperosmolar saline on subsequent challenge by MBS in our study may be because the hyperosmolar solution caused minor bronchoconstriction only.

The two solutions causing most inhibition were the most acidic (L-aspirin) and the most alkaline (indomethacin), which suggests that pH was not a major determinant of the protection seen.

Thus, inhaled L-aspirin and indomethacin have broadly similar effects against MBS and AMP induced bronchoconstriction in the doses given, although the effect of indomethacin on MBS was not quite statistically significant. The osmolarity and pH of the solutions did not appear to be important determinants of the response. The effect of L-aspirin and indomethacin is likely to be due to cyclooxygenase inhibition which reduces the production of contractile prostaglandins during MBS and AMP challenge.

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9 Pieroni M, Vaghi A, Refini RM. Inhaled indomethacin partially prevents the late but not the early bronchial allergic reaction. Am Rev Respir Dis 1993;147:25A.


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