Nebulised salbutamol administered during sputum induction improves bronchoprotection in patients with asthma

M Delvaux, M Henket, L Lau, P Kange, P Bartsch, R Djukanovic, R Louis

Background: Inhalation of hypertonic or even isotonic saline during sputum induction may cause bronchospasm in susceptible patients with asthma, despite premedication with 400 μg inhaled salbutamol delivered by pressurised metered dose inhaler (pMDI). The bronchoprotection afforded by additional inhaled salbutamol administered through the ultrasonic nebuliser during sputum induction was investigated.

Methods: Twenty patients with moderate to severe asthma underwent sputum induction by inhaling saline 4.5% (or 0.9% if post-bronchodilatation forced expiratory volume in 1 second (FEV₁) < 65% predicted) for 10 minutes according to two protocols given 1 week apart in random order. At visit A the patients received 400 μg salbutamol administered through a pMDI + spacer 20 minutes before induction while at visit B the premedication was supplemented by 1500 μg nebulised salbutamol inhaled throughout the induction procedure. Both the investigator and the patients were blind to the nebulised solution used. FEV₁ was recorded during sputum induction at 1, 3, 5, and 10 minutes. Sputum cell counts and histamine, tryptase and albumin levels in the supernatants were determined.

Results: The mean (SE) maximal reduction in FEV₁ over the 10 minute period of sputum induction was 11.7 (2.8)% at visit A, which was significantly greater than at visit B (2.6 (1.2)%; mean difference 9% (95% CI 2.7 to 15.4), p=0.01). Total and differential sputum cell counts as well as albumin, tryptase, and histamine levels did not differ between the two visits.

Conclusion: The addition of inhaled salbutamol through an ultrasonic nebuliser markedly improves bronchoprotection against saline induced bronchoconstriction in patients with moderate to severe asthma undergoing sputum induction without affecting cell counts and inflammatory markers.
randomised to receive either inhaled salbutamol 400 μg administered through pMDI and Volumatic (Glaxo Smith Kline, UK) 20 minutes before sputum induction (visit A) or the same regimen + additional nebulised salbutamol 1571 μg during the sputum induction procedure (visit B). Both the asthmatic subjects and the laboratory function technician who performed sputum induction and spirometric tests were blind to the inhaled solution used. The two sputum inductions were separated by a period of 1 week and were performed at the same time of the day (±2 hours). There was no change in the maintenance treatment between inductions and subjects were allowed to take their usual maintenance drugs (including long acting β2 agonists) on the induction. Importantly, the mean consumption of short acting bronchodilator during the preceding week and the baseline lung function values before sputum induction were similar between visits A and B (table 2). FEV₁ was measured as the best of three flow-volume curves using an electronic spirometer (Spirobank, MIR, Rome, Italy) before and 15 minutes after 400 μg inhaled salbutamol, the latter value being considered as the baseline value.

Sputum induction was started with either hypertonic or isotonic saline according to post-bronchodilator FEV₁. Patients with an FEV₁ ≥65% predicted inhaled 4.5% saline while those with FEV₁ <65% predicted were given 0.9% saline instead. Two patients had a post-bronchodilator FEV₁ slightly less than 65% predicted at visit A (62% and 64% predicted, respectively) but more than 65% at visit B (66% and 67% predicted, respectively). As visit A was the first visit for these two patients, they also inhaled isotonic saline at their second visit (visit B) for consistency.

### Sputum processing
Sputum induction was performed at the same time of the day (±2 hours). There was no change in the maintenance treatment between inductions and subjects were allowed to take their usual maintenance drugs (including long acting β2 agonists) on the induction. Importantly, the mean consumption of short acting bronchodilator during the preceding week and the baseline lung function values before sputum induction were similar between visits A and B (table 2). FEV₁ was measured as the best of three flow-volume curves using an electronic spirometer (Spirobank, MIR, Rome, Italy) before and 15 minutes after 400 μg inhaled salbutamol, the latter value being considered as the baseline value.

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### Biochemical assays
Albumin was measured by rocket electrophoresis as previously described. Histamine was measured by RIA (Immunotech, Marseille, France) with a sensitivity of 0.02 ng/ml. Tryptase was measured by an immunoassay (UniCAP, Pharmacia, Uppsala, Sweden) with a sensitivity of 1 ng/ml.

### Statistical analyses
Changes in FEV₁ during sputum induction were expressed as mean (SE), and sputum cell counts and biochemical markers as median (range). Assessment of the significance of a bronchoconstriction during the sputum induction for each procedure was performed using a one sample Student’s t test. Comparisons of the maximal falls in FEV₁ between the two procedures were performed by a paired Student’s t test. Comparisons of sputum cell counts and biochemical markers between the two procedures were performed by the Wilcoxon rank sum test, whereas reproducibility was assessed by calculating the intraclass coefficient of correlation as previously

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**Table 1** Demographic and functional characteristics of study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit A (n = 20)</th>
<th>Visit B (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>44 (23–82)</td>
<td>44 (23–82)</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>11/9</td>
<td>11/9</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>6/20</td>
<td>6/20</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV₁ (ml)*</td>
<td>2337 (1030–3670)</td>
<td>2306 (1120–3610)</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV₁ (% pred)*</td>
<td>73 (38–132)</td>
<td>73 (44–136)</td>
</tr>
<tr>
<td>Post-bronchodilator FEV₁ (ml)*</td>
<td>2554 (1130–3500)</td>
<td>2651 (1170–3680)</td>
</tr>
<tr>
<td>Reversibility (%)*</td>
<td>13 (–15–59)</td>
<td>17 (–2–70)</td>
</tr>
<tr>
<td>Post-bronchodilator FEV₁ (% pred)*</td>
<td>80 (40–143)</td>
<td>82 (40–142)</td>
</tr>
<tr>
<td>Post-bronchodilator FEV₁ ≥65%</td>
<td>14/20</td>
<td>16/20</td>
</tr>
<tr>
<td>Post-bronchodilator FEV₁ &lt;65%</td>
<td>6/20</td>
<td>4/20</td>
</tr>
</tbody>
</table>

*Age and lung function results are expressed as mean (range).

+Visit A: premedication with inhaled salbutamol 400 μg pMDI. Visit B: premedication with inhaled salbutamol 400 μg pMDI + nebulised salbutamol 1.500 μg.

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**Table 2** Treatment characteristics of study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Visit A (n = 20)*</th>
<th>Visit B (n = 20)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short acting β2 agonists (puffs/week)</td>
<td>16 (0–70)</td>
<td>14 (0–64)</td>
</tr>
<tr>
<td>Treated with long acting β2 agonists (n)</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Treated with inhaled corticosteroids (n)</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Treated with theophylline (n)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Treated with oral corticosteroids (n)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>No controller medication (n)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*Visit A: premedication with inhaled salbutamol 400 μg pMDI; visit B: premedication with inhaled salbutamol 400 μg pMDI + nebulised salbutamol 1.500 μg.
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Salbutamol). Distribution of the maximal falls in forced expiratory volume in 1 second (FEV1) at visit A (NaCl) and visit B (NaCl + nebulised salbutamol).

**RESULTS**

There was no significant difference between post-bronchodilator FEV1 values measured before starting sputum induction on visits A and B (table 1). All the subjects inhaled saline for 10 minutes on both occasions. Sputum induction caused significant bronchospasm that peaked at 10 minutes and reached 10.4 (3.2)% (p < 0.01) when subjects were premedicated with 400 μg salbutamol inhaled through a pMDI and spacer (visit A). In contrast, inhalation of the same amount of saline did not produce any significant fall in FEV1 when the patients received nebulised salbutamol in addition to conventional premedication (visit B, fig 1). The maximal fall in FEV1 over the 10 minute period was 11.7 (2.8)% at visit A and 2.6 (1.2)% at visit B (fig 2). The mean (95% CI) difference for the maximal fall in FEV1 between visits A and B was 9.2 (2.7 to 15.4), p < 0.01. Six of the 20 patients had a fall in FEV1 of > 20% at 10 minutes when premedicated with the conventional procedure while none reached this threshold when receiving nebulised salbutamol.

At visit A the mean maximal fall in FEV1 was 11.1 (3.6)% (p < 0.05, one sample t test) in patients who inhaled hypertonic saline and 13.2 (4.9)% (p < 0.05) in patients who inhaled isotonic saline (post-bronchodilator FEV1 < 65% predicted). The magnitude of the falls in FEV1 with the conventional procedure was inversely related to baseline FEV1 (r = −0.46, p < 0.05) and directly related to both the extent of bronchodilation after inhaled salbutamol 400 μg (r = 0.58, p < 0.01) and the consumption of β2 agonist in the preceding week (r = 0.44, p = 0.05).

At visit A, patients who were regularly treated with long acting β2 agonists (n = 11) had a fall in FEV1 of 11.2 (3.8)% compared with 12.5 (4.5)% in those not treated with long acting β2 agonists (n = 9). When receiving nebulised salbutamol, the fall in FEV1 was 2.4 (1.7)% in patients on regular treatment with long acting β2 agonists and only 3 (1.6)% in patients not so treated (fig 3).

Mean (SE) FEV1 values measured 10 and 20 minutes after the end of sputum induction at visit A were 7.1 (1.9)% and 4.3 (2.4)% respectively. Two subjects still had a fall in FEV1 of more than 20% by this time which was quickly reversed to less than 5% of baseline by nebulised salbutamol (2500 μg) and ipratropium bromide (250 μg). At visit B there was no late bronchoconstriction following sputum induction since mean FEV1 values remained above 95% of baseline at 10 and 20 minutes.

Although 18 patients produced an adequate sputum sample on at least one occasion, only 15 produced readable samples on both occasions allowing for comparison between the two procedures. Sputum induction failed in two patients at visit A and in two others at visit B. One patient was unable to produce any sample at any visit. In the patients who produced two adequate samples there was no significant difference between sputum cell counts and sputum albumin, tryptase and histamine levels measured at either visit (table 3).

**DISCUSSION**

This study shows that the addition of salbutamol 1500 μg in the ultrasonic nebuliser during sputum induction reinforces the bronchoprotection obtained with 400 μg inhaled salbutamol through pMDI in patients with moderate to severe asthma. Improved bronchoprotection is observed irrespective of current treatment with long acting β2 agonists. In addition, inhalation of a high dose of salbutamol does not modify cellular and mediator activity in the airway inflammatory process as evidenced by sputum cell counts, and albumin, tryptase and histamine levels.

Our data confirm that inhaling hypertonic and even isotonic saline carries the risk of significant bronchospasm in moderate to severe asthmatics despite premedication with salbutamol 400 μg delivered by pMDI, which highlights the need to improve the safety of the procedure. We have shown...
that complementary salbutamol (1500 µg) inhaled through an ultrasonic nebuliser markedly reduces the intensity of bronchospasm seen in patients with moderate to severe asthma undergoing sputum induction. None of the 20 patients studied had a fall in FEV₁ of more than 20% during sputum induction when receiving nebulised salbutamol compared with six patients who had a fall in FEV₁ of more than 20% (range 20.3–37.2%) at the end of sputum induction when only receiving 400 µg inhaled salbutamol through pMDI. In our study the rate of significant bronchospasm (30%) with standard premedication is slightly higher than that reported by Fahy et al. (14%) in a recent multicentre study of asthmatics whose baseline FEV₁ values were very similar to those of our subjects.11

In agreement with previous studies,12 13 we found that both baseline FEV₁ values and β₂ agonist consumption are parameters that may influence the magnitude of bronchospasm following inhalation of saline. It is therefore worth noting that these two parameters were similar between visits A and B, making comparison between the two study days valid. The relationship between the dose of inhaled salbutamol and the extent of bronchoprotection has only been studied over a narrow range of concentrations so far. Bel and colleagues14 showed that salbutamol 200 and 400 µg inhaled through a pMDI were both very efficient in shifting to the right the concentration of histamine or methacholine provoking a fall in FEV₁ of 20% or more (PC₂₀) with a small dose related effect for PC₂₀ histamine but not for PC₂₀ methacholine.14 Inhalation of salbutamol 100–400 µg by MDI produces strong bronchoprotection against methacholine that is, however, weakly proportional to the dose since 80% of protection is already achieved with 100 µg.15 On the other hand, inhalation of 1600 µg salbutamol dry powder on top of regular treatment with salmeterol (50 µg twice daily) or formoterol (12 µg twice daily) did not increase the bronchoprotection towards methacholine. The inability of a dose of salbutamol similar to the one we used and administered through a dry powder inhaler to further improve the bronchoprotection given by long acting β₂ agonists16 therefore suggests that, in our study, the ultrasonic nebulisation itself may well reinforce the bronchoprotection obtained with salbutamol.

By showing no difference in the magnitude of bronchospasm induced by saline inhalation between patients with and without long acting β₂ agonists, our results indicate that it would be unwise to consider that asthmatic patients are always protected against saline induced bronchoconstriction when regularly treated with long acting β₂ agonists. The tolerance in bronchoprotection produced by chronic usage of β₂ agonists may be involved.17 However, it should be kept in mind that the patients in our study who received long acting β₂ agonists also suffered from more severe asthma, exhibiting the poorest baseline lung function, and were therefore the most likely to show a dramatic change in FEV₁ after saline.

It is interesting that the delivery of high dose salbutamol into the Airways does not alter the profile of sputum cells nor its albumin, tryptase and histamine content. Inhalation of hypertonic saline may trigger mast cell degranulation18 and favour vascular leakage19 into the Airways. On the other hand, there is evidence in the literature that β₂ agonists can prevent mast cell degranulation and inhibit plasma exudation.7 As these two properties may theoretically partly contribute to the bronchoprotective effect of β₂ agonists,20 we wished to determine whether inhalation of a high dose of salbutamol, compared with a low dose, has any effect on the sputum content of mast cell derived mediators and a marker of plasma exudation. Our results show that high dose salbutamol had no effect on the levels of histamine, tryptase, or albumin. This suggests that the greater bronchoprotective effect of high dose salbutamol is neither related to mast cell stabilisation nor to the anti-exudative effect during sputum induction.

This study shows that additional inhalation of salbutamol through an ultrasonic nebuliser improves the safety of sputum induction in patients with moderate to severe asthma without altering its cellular and biochemical content. We believe that this may be of practical importance to the investigation of Airways inflammation in patients with moderate to severe asthma.

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REFERENCEs

### Table 3 Sputum cell counts and biochemical markers

<table>
<thead>
<tr>
<th></th>
<th>Visit A</th>
<th>Visit B</th>
<th>p value</th>
<th>ICCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cells (%)</td>
<td>5 (1–46)</td>
<td>6 (0–52)</td>
<td>&gt;0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Non-squamous cells (×10³/g)</td>
<td>1.8 (0.3–37.8)</td>
<td>1.5 (0.3–15.5)</td>
<td>&gt;0.05</td>
<td>0.41</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>68 (46–94)</td>
<td>72 (36–84)</td>
<td>&gt;0.05</td>
<td>0.53</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>35 (1–74)</td>
<td>23 (2–92)</td>
<td>&gt;0.05</td>
<td>0.61</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>0.4 (0–2.4)</td>
<td>1 (0.2–2.6)</td>
<td>&gt;0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>47 (13–99)</td>
<td>55 (1–97)</td>
<td>&gt;0.05</td>
<td>0.82</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1 (0–35)</td>
<td>2 (0–48)</td>
<td>&gt;0.05</td>
<td>0.74</td>
</tr>
<tr>
<td>Epithelial cells (%)</td>
<td>4 (0–27)</td>
<td>3 (0–15)</td>
<td>&gt;0.05</td>
<td>0.52</td>
</tr>
<tr>
<td>Albumin (µg/l)</td>
<td>152 (43–380)</td>
<td>133 (54–231)</td>
<td>&gt;0.05</td>
<td>0.09</td>
</tr>
<tr>
<td>Tryptase (ng/ml)</td>
<td>1.29 (0.31–7.32)</td>
<td>1.36 (0.34–3.66)</td>
<td>&gt;0.05</td>
<td>0.65</td>
</tr>
<tr>
<td>Histamine (ng/ml)</td>
<td>3.5 (1.1–66)</td>
<td>4.9 (0.8–61)</td>
<td>&gt;0.05</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Results are expressed as median (range). $p$ values indicates the value of the Wilcoxon rank sum test between visits A and B. ICCC = intraclass coefficient of correlation.
Higher tuberculosis mortality in India seen in those who have ever smoked


This case-control study examined the smoking habits and medical causes of mortality in men from southern India. The death rates of current or former smokers were double those of never smokers (RR = 2.1 (95% CI 2.0 to 2.2)). Of this excess mortality among smokers, a third involved respiratory disease. Tuberculosis was the chief cause of respiratory mortality (4.5 (95% CI 4.0 to 5.0), smoking attributed fraction 61%). A separate survey of 250 000 men living in the urban study area found that smokers are three times more likely than never smokers to report a history of tuberculosis, corresponding to a higher rate of progression of chronic subclinical infection to clinical disease. The authors conclude that smoking, which is a cause of half the deaths from tuberculosis in men in India, increases the incidence of clinical tuberculosis.

This and similar studies will no doubt stimulate research into the mechanisms behind reactivation of tuberculosis by smoking. Some clues may be derived from the reported effects of smoking on other diseases. Smokers have a reduced incidence of sarcoidosis and extrinsic allergic alveolitis, diseases in which tumour necrosis factor-α (TNF-α) plays a key role. Moreover, the use of anti-TNF-α agents for conditions such as rheumatoid arthritis leads to reactivation of tuberculosis. Collectively, these data converge on the hypothesis that smoking might oppose the release or function of TNF-α in the lungs. Recent work on the effects of nicotine has revealed likely pathways (Wang H, et al. Nature 2003;421:384–8; Borovikova LV, et al. Nature 2000;405:458–62).

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