

ASTHMA

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

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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-bronchodilation 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.

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Since the first description of sputum induction by Pin *et al* in 1992,¹ the technique has been widely used in asthma research. Although several studies have reported on the methodology of sputum induction,² some outstanding issues with respect to the safety of the procedure remain. Inhalation of hypertonic saline carries the risk of severe bronchoconstriction in patients with bronchial hyperresponsiveness,³ and hypertonic saline challenge is a recognised indirect challenge to assess bronchial hyperresponsiveness in asthma.⁴ This has led to the recommendation of using inhaled salbutamol 200–400 µg before sputum induction in patients with asthma unless measurement of bronchial responsiveness to saline is an outcome of the study.⁵ In most studies inhaled salbutamol has been delivered by pressurised metered dose inhaler (pMDI), some authors combining the pMDI with a spacer to improve lung deposition. Despite this type of premedication, some asthmatic patients still experience breakthrough bronchoconstriction. One way to reduce the risk of bronchoconstriction is to induce sputum by inhalation of isotonic rather than hypertonic saline, as advocated by Pizzichini *et al* for severe asthmatic exacerbations.⁶ However, even isotonic saline may be sufficiently irritating to the airways to trigger bronchospasm in asthmatic patients with very reactive airways. The dose of salbutamol and the optimal way to deliver it remains an issue for the safety of sputum induction.⁵

We hypothesised that delivering salbutamol via ultrasonic nebuliser together with isotonic or hypertonic saline may provide better bronchoprotection than inhaled salbutamol pMDI 400 µg alone administered through a Volumatic chamber. We have assessed 20 patients with moderate to severe asthma and compared the falls in forced expiratory volume in 1 second (FEV₁) that occurred during sputum

induction when subjects were premedicated with either salbutamol pMDI (400 µg) or salbutamol pMDI (400 µg) + nebulised salbutamol delivered throughout the induction procedure. To ensure that nebulised salbutamol does not affect the key indicators in the sputum we compared the two induction procedures with respect to sputum cell counts and several biochemical markers—albumin as an indicator of plasma exudation and tryptase and histamine as markers of mast cell activation—two activities that may theoretically be influenced by β₂ agonists.⁷

METHODS

Subjects

Twenty patients with moderate to severe asthma diagnosed according to ATS criteria⁸ were recruited from outpatient clinics. Three had been admitted to hospital and five others had received a course of systemic corticosteroids for a severe asthma exacerbation during the previous year. The demographic and functional characteristics of the subjects are shown in table 1.

Sixteen subjects received regular treatment with inhaled corticosteroids, 11 of whom were also receiving inhaled long acting β₂ agonists. Two subjects were also on oral corticosteroids with a daily dose of 16 and 32 mg methylprednisolone, respectively. Maintenance treatment was kept constant over the 4 weeks preceding the study (table 2). The protocol was approved by the local ethical committee and volunteers gave their written informed consent.

Study design

This was a randomised crossover study. There was a run in period during which subjects recorded their daily consumption of relief bronchodilators into diary cards. Subjects were

Table 1 Demographic and functional characteristics of study subjects

	Visit A (n=20)†	Visit B (n=20)†
Age (years)*	44 (23–82)	44 (23–82)
Sex (F/M)	11/9	11/9
Smoking habit	6/20	6/20
Pre-bronchodilator FEV ₁ (ml)*	2337 (1030–3670)	2306 (1120–3610)
Pre-bronchodilator FEV ₁ (% pred)*	73 (38–132)	73 (44–136)
Post-bronchodilator FEV ₁ (ml)*	2554 (1130–3500)	2651 (1170–3680)
Reversibility (%)*	13 (–15–59)	17 (–2–70)
Post-bronchodilator FEV ₁ (% pred)*	80 (40–143)	82 (40–142)
Post-bronchodilator FEV ₁ ≥65%	14/20	16/20
Post-bronchodilator FEV ₁ <65%	6/20	4/20

*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 1500 µg.

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 β_2 agonists) on the morning of 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 induction

Saline was inhaled through an ultrasonic nebuliser (Devilbiss 2000), the mean output of which was calculated to be 0.93 ml/min. The cup of the nebuliser was filled with 50 ml

hypertonic/isotonic saline to which was added 1.75 ml salbutamol solution at 5 mg/ml. The dose of nebulised salbutamol was dependent on the duration of sputum induction and was calculated by multiplying the concentration of salbutamol in the cup of the nebuliser (169 µg/ml) by the output of the nebuliser (0.93 ml/min) and the duration of the induction. FEV₁ was measured at 1, 3, 5, and 10 minutes after starting inhalation. Inhalation of saline was stopped after 10 minutes or when a fall in FEV₁ of >20% from baseline had occurred. After performing spirometric measurements at 5 and 10 minutes the subjects were asked to rinse their mouth with tap water and to cough up sputum into a plastic container. For safety reasons, FEV₁ was measured 10 and 20 minutes after the end of the induction in every patient. Two subjects who still had a fall in FEV₁ of >20% at this time received additional nebulised salbutamol and ipratropium bromide and were kept under observation until their FEV₁ value had returned to within 5% of baseline.

Sputum processing

Samples were poured into a 50 ml polypropylene tube, weighed, and diluted with a threefold weight of a phosphate buffered saline (PBS) solution for homogenisation. The samples were then rocked at room temperature for 20 minutes and centrifuged at 400 g for 10 minutes at 4°C. The supernatant was stored at –80°C until biochemical analyses for albumin and histamine. The cellular phase was dispersed in 1 ml PBS without Ca²⁺ and Mg²⁺ solution for total cell counts using a manual haemocytometer. The differential cell count was performed on cytopins stained with Diff-Quick by counting 500 cells under a light microscope.

Biochemical assays

Albumin was measured by rocket electrophoresis as previously described.⁹ 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

Table 2 Treatment characteristics of study subjects

	Visit A (n=20)*	Visit B (n=20)*
Short acting β_2 agonists (puffs/week)	16 (0–70)	14 (0–64)
Treated with long acting β_2 agonists (n)	11	11
Treated with inhaled corticosteroids (n)	16	16
Treated with theophylline (n)	1	1
Treated with oral corticosteroids (n)	2	2
No controller medication (n)	3	3

*Visit A: premedication with inhaled salbutamol 400 µg pMDI; visit B: premedication with inhaled salbutamol 400 µg pMDI + nebulised salbutamol 1500 µg.

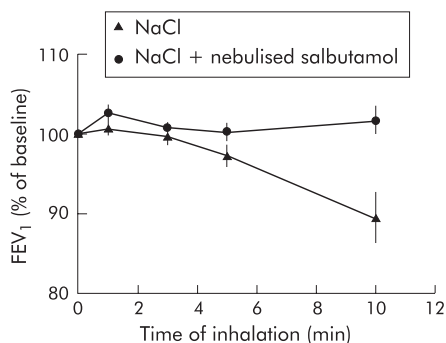


Figure 1 Time course of bronchoconstriction during sputum induction by inhalation of hypertonic or isotonic saline in 20 patients with moderate to severe asthma. Results expressed as mean (SE) values.

described.¹⁰ p values of <0.05 were considered statistically significant. Given the repeatability of the functional and biochemical parameters and setting a risk error α at 0.05, our sample size had a power of 98%, 96%, 82%, and 84% to detect a 50% inhibition in the fall in FEV₁ and of the sputum levels of albumin, histamine and tryptase, respectively.

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 μ g 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% (2.7 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

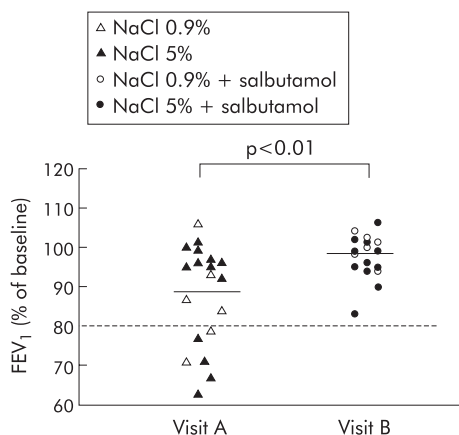


Figure 2 Distribution of the maximal falls in forced expiratory volume in 1 second (FEV₁) at visit A (NaCl) and visit B (NaCl + nebulised salbutamol).

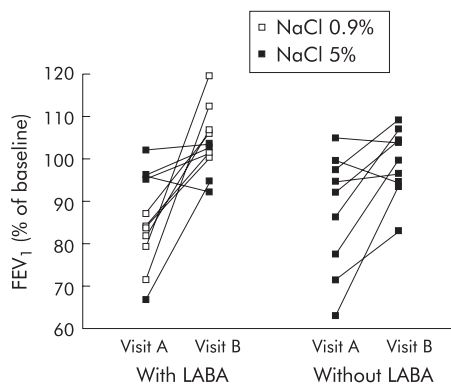


Figure 3 Comparison of the maximal falls in FEV₁ between patients treated and not treated with long acting β_2 agonists (LABA).

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 μ g ($r = 0.58$, $p<0.01$) and the consumption of β_2 agonist in the preceding week ($r = 0.44$, $p = 0.05$).

At visit A, patients who were regularly treated with long acting β_2 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 β_2 agonists ($n = 9$). When receiving nebulised salbutamol, the fall in FEV₁ was 2.4 (1.7)% in patients on regular treatment with long acting β_2 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 μ g) and ipratropium bromide (250 μ g). 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 μ g in the ultrasonic nebuliser during sputum induction reinforces the bronchoprotection obtained with 400 μ g inhaled salbutamol through pMDI in patients with moderate to severe asthma. Improved bronchoprotection is observed irrespective of current treatment with long acting β_2 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 μ g delivered by pMDI, which highlights the need to improve the safety of the procedure. We have shown

Table 3 Sputum cell counts and biochemical markers

	Visit A	Visit B	p value	ICCC
Squamous cells (%)	5 (1–46)	6 (0–52)	>0.05	0.04
Non-squamous cells ($\times 10^6/g$)	1.8 (0.3–37.8)	1.5 (0.3–15.5)	>0.05	0.41
Viability (%)	68 (46–94)	72 (36–84)	>0.05	0.53
Macrophages (%)	35 (1–74)	23 (2–92)	>0.05	0.61
Lymphocytes (%)	0.4 (0–2.4)	1 (0.2–2.6)	>0.05	0.10
Neutrophils (%)	47 (13–99)	55 (1–97)	>0.05	0.82
Eosinophils (%)	1 (0–35)	2 (0–48)	>0.05	0.74
Epithelial cells (%)	4 (0–27)	3 (0–15)	>0.05	0.52
Albumin ($\mu g/l$)	152 (43–380)	133 (54–231)	>0.05	0.29
Tryptase	1.29 (0.31–7.32)	1.36 (0.34–3.66)	>0.05	0.65
Histamine (ng/ml)	3.5 (1.1–66)	4.9 (0.8–61)	>0.05	0.77

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.

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 β_2 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 β_2 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 β_2 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 β_2 agonists. The tolerance in bronchoprotection produced by chronic usage of β_2 agonists may be involved.¹⁷ However, it should be kept in mind that the patients in our study who received long acting

β_2 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 β_2 agonists can prevent mast cell degranulation and inhibit plasma exudation.⁷ As these two properties may theoretically partly contribute to the bronchoprotective effect of β_2 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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LUNG ALERT

Higher tuberculosis mortality in India seen in those who have ever smoked

▲ Gajalakshmi V, Peto R, Santhanakrishna T, Jha P. Smoking and mortality from tuberculosis and other diseases in India: retrospective study of 43 000 adult male deaths and 35 000 controls. *Lancet* 2003;**362**:507–15.

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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