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Comparison of the efficacy of preservative free ipratropium bromide and Atrovent nebuliser solution

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ABSTRACT The paradoxical bronchoconstriction observed with commercially available isotonic ipratropium bromide nebuliser solution (Atrovent) in patients with asthma results from an adverse reaction to the preservatives, benzalkonium chloride and ethylenediaminetetra-acetic acid (EDTA). The airway response to inhaled Atrovent and preservative free ipratropium bromide nebuliser solutions has been examined in a double blind study. On separate occasions 30 asthmatic subjects inhaled 2 ml of the solutions and airway calibre was measured in terms of FEV_1 for 45 minutes. Atrovent nebuliser solution provoked a greater than 20% fall in FEV_1 in five of the 30 subjects, whereas this did not occur after preservative free ipratropium bromide. Inhalation of the preservative free solution resulted in more rapid and greater overall bronchodilatation than Atrovent, with mean maximum increases in FEV_1 of 29·2% and 18·5% respectively. It is concluded that the risk of paradoxical bronchoconstriction with ipratropium bromide is considerably reduced by removal of benzalkonium chloride and EDTA and that preservative free ipratropium bromide is a more potent bronchodilator than the currently available Atrovent solution.

Introduction

Ipratropium bromide (Atrovent) administered for inhalation by pressurised metered dose aerosol has found wide use for the treatment of reversible obstructive airways disease. The drug is also available as a solution for nebulisation in patients with severe airflow limitation, though occasional paradoxical bronchoconstriction has been reported.[1-3] When the bronchoconstriction had been shown to be largely accounted for by the hypotonicity of the original nebulised solution[4] this was reformulated as an isotonic solution. Despite this change occasional reports of bronchoconstriction still occur.[5]

In a single case study Patel *et al*[5] suggested that bronchoconstriction with ipratropium bromide occurred as an idiosyncratic response to the bromide ion, but in a controlled study in patients who developed bronchoconstriction with hypotonic ipratropium bromide and sodium bromide administration of the solutions in the isotonic form failed to produce this response.[6] In a further double blind, placebo controlled study in asthmatic subjects we have shown that both benzalkonium chloride and ethylenediaminetetra-acetic acid (EDTA), present in isotonic Atrovent as bacteriostatic and stabilising agents, cause bronchoconstriction.[7] Benzalkonium chloride caused bronchoconstriction at the concentration present in the nebuliser solution of 0·25 g/l, whereas EDTA caused bronchoconstriction only at concentrations more than twice that present in the nebuliser solution (0·5 g/l). In this study about a quarter of asthmatic subjects who inhaled Atrovent from a starting volume of 4 ml (that is, 1 mg ipratropium bromide) developed immediate bronchoconstriction with a fall in forced expiratory volume in one second (FEV_1) of more than 20% from baseline. When these subjects inhaled only isotonic preservative free ipratropium bromide bronchodilatation occurred. We have now investigated the effect of the maximum single dose of isotonic Atrovent recommended by the manufacturer (2 ml—that is, 0·5 mg ipratropium bromide) on airway calibre in patients with asthma and chronic bronchitis and compared its bronchodilator effect with that of preservative free ipratropium bromide.

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Methods

PATIENTS

Thirty patients took part in the study and their characteristics are described in table 1. All had

Table 1 *Characteristics of the patients*

	All patients	*Atopic*	*Non-atopic*
Number	30	13	17
Age (mean (SEM))	54·7 (2·7)	47·5 ± 4·1	60·1 ± 2·9
Sex (M:F)	21:9	8:5	14:4
No of smokers	22	8	13
No with chronic bronchitis*	15	3	12
FEV_1 (% predicted, mean (SEM))	62·4 (5·3)	73·7 (9·6)	53·8 (4·5)
PC_{20} methacholine (mg/ml)†: mean (range)	0·31 (0·04 – 3·0)	0·24 (0·06 – 1.2)	0·36 (0·04 – 3·0)

*Medical Research Council definition.
†Geometric mean.

documented reversible airways obstruction, having previously shown a greater than 15% improvement in FEV_1 15 minutes after inhalation of 200 μg of salbutamol. Twenty two of the patients were smokers, and 15 conformed to the Medical Research Council (MRC) definition of chronic bronchitis with chronic mucus hypersecretion, all being past or current smokers. All patients were receiving regular inhaled β_2 adrenoceptor agonists with or without inhaled corticosteroids or inhaled ipratropium bromide or both; these were withheld for 12 hours before each visit to the laboratory. The study was approved by the Southampton University Hospitals ethical subcommittee and written informed consent was obtained from each patient.

STUDY DESIGN

Subjects attended the laboratory on three separate occasions at least 48 hours apart. During the first two visits the patients received 2 ml of either isotonic Atrovent nebuliser solution containing benzalkonium chloride 0·25 g/l and EDTA 0·5 g/l or isotonic preservative free ipratropium bromide by inhalation double blind and in random order. The solutions were nebulised at room temperature by compressed air with an Inspiron nebuliser (Bard, Sunnywell, Sunderland) with an output of 0·4 ml/min at a flow rate of 8 l/min. The aerosol was inhaled through a mouthpiece. Two baseline measurements of FEV_1 were made followed by inhalation of saline vehicle solution as five maximal inhalations from functional residual capacity. If the FEV_1 had not fallen by more than 10% from baseline after three minutes Atrovent or preservative free ipratropium bromide solutions were administered during tidal breathing from a starting volume of 2 ml with nebulisation to dryness. FEV_1 was measured immediately and at 2, 5, 10, 15, 30, and 45 minutes after nebulisation. On the third visit the 26 patients whose baseline FEV_1 was greater than 1·0 litres underwent inhalation challenge with methacholine modified from the method described by Chai.[8] Skin-prick tests with six common allergens (house dust, *Dermatophagoides pteronyssinus*, cat, dog, feather, grass pollen, and a control solution—Bencard, Middlesex) were performed on all patients. A weal diameter of over 4 mm at 10 minutes in response to one or more allergens was considered a sign of atopy.

DATA ANALYSIS

Baseline FEV_1 values were compared between study days by means of Student's *t* test for paired data. FEV_1 after nebulisation was expressed as a percentage of the post-saline baseline value and plotted against time. The airways response for the two ipratropium bromide solutions was assessed by comparing the maximum and minimum values of FEV_1 achieved, and the areas under the FEV_1–time course curves (AUC) calculated by trapezoid integration. For each of the three variables comparisons were made between Atrovent nebuliser solution and preservative free ipratropium bromide, paired Student's *t* tests being used for (*a*) all patients and (*b*) subgroups of patients divided on the basis of the presence or absence of atopy, smoking history, and the presence or absence of chronic bronchitis. Multiple linear regression analysis was used to investigate any relation between the airway response to the nebulised solutions and atopy, smoking, and chronic bronchitis.

The provocation concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) was derived from the concentration-response curve by linear interpolation. The relation between methacholine responsiveness (PC_{20} methacholine) and the airway response to Atrovent and preservative free ipratropium bromide solutions were determined by linear regression analysis. Minitab[9] was used for all statistical analyses.

Results

All patients completed the study. There was no significant difference between mean baseline FEV_1 on different treatment days. Inhalation of isotonic Atrovent caused initial bronchoconstriction with a fall in FEV_1 of over 20% from baseline in five of the 30 subjects. Although nebulised Atrovent solution caused progressive bronchodilatation in most subjects (fig 1, table 2), the FEV_1 remained below the initial baseline value 45 minutes after inhalation in six subjects.

After inhalation of 2 ml preservative free ipratropium bromide one subject showed an initial fall in FEV_1 of 19%, which returned to within 2% of baseline by 2 minutes. None of the other subjects showed a fall in FEV_1 of more than 10%. All subjects subsequently achieved bronchodilatation with this preparation (fig 1). The speed of onset and the degree of bronchodilatation was greater after preservative free ipratropium bromide than after Atrovent (fig 1, table 2).

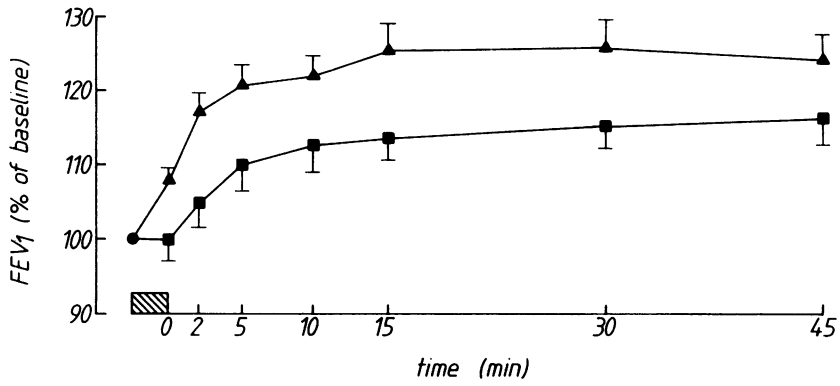

Fig 1 *Effect of Atrovent (squares) and preservative free ipratropium bromide (triangles) on airway calibre in 30 subjects: means with standard errors.*

Bronchial responsiveness to inhaled methacholine was assessed in 26 subjects. There was no significant correlation between the log PC_{20} FEV_1 methacholine and the airway response to either Atrovent or the preservative free solution.

There were no significant differences between smokers and non-smokers or between bronchitic and non-bronchitic patients in their response to either Atrovent or preservative free ipratropium bromide. There was, however, a difference in response between atopic and non-atopic subjects (fig 2, table 3). After Atrovent three of the 13 atopic subjects developed a fall in FEV_1 of over 20% from baseline, compared with only two of 17 non-atopic subjects. Overall, Atrovent provoked a temporary fall (of 8·3 l) in FEV_1 in atopic subjects, which was not seen in the non-atopic group ($p < 0·05$). Multiple linear regression confirmed that atopy was the strongest determinant of the bronchoconstrictor response to Atrovent solution. The difference between groups could not be attributed to the level of bronchial responsiveness, with geometric mean PC_{20} FEV_1 methacholine values of 0·24 and 0·36 mg/ml for the atopic and non-atopic subjects, a non-significant difference.

Discussion

This study shows that inhalation of 2 ml isotonic Atrovent nebuliser solution, the maximum single dose recommended by the manufacturers, causes bronchoconstriction in an appreciable proportion of patients with reversible obstructive airways disease. In this group of patients selected at random from the outpatient department, five out of 30 patients had substantial bronchoconstriction, defined as a fall in FEV_1 of over 20% from baseline. This compares with a previous study in which just over one in four patients with asthma had a similar degree of bronchoconstriction when challenged with 4 ml Atrovent solution. In the present study all patients had previously shown reversibility of their airways obstruction by an improvement in FEV_1 of over 15% with a β_2 adrenoreceptor agonist, and half also had symptoms conforming to the MRC definition of chronic bronchitis with mucus hypersecretion. Nebulised Atrovent caused bronchoconstriction in a similar proportion of patients with and without chronic bronchitis, and the changes were similar in magnitude to those observed in patients with more classical asthma without mucus hypersecretion.

After inhalation of preservative free ipratropium bromide appreciable bronchoconstriction was observed in only one subject and this was transient. In the absence of preservatives ipratropium bromide also proved more effective than Atrovent in producing bronchodilatation throughout the 45 minutes of the study, achieving almost double the change in FEV_1 observed with Atrovent. The response to preservative free ipratropium bromide occurred more rapidly than

Table 2 *Airway responses (means with standard errors in parentheses) to nebulised Atrovent and preservative free ipratropium bromide (PFIB) in the 30 patients*

	Atrovent	*PFIB*	*Difference (PFIB—Atrovent)*	*p**
Highest FEV_1 after nebulisation (% change from baseline)	18·5 (2·8)	29·2 (3·6)	10·7 (2·9)	< 0·001
Lowest FEV_1 after nebulisation (% change from baseline)	−3·5 (2·2)	5·5 (2·0)	9·0 (2·5)	< 0·004
Area under the FEV_1-time course curve	565 (119)	1030 (145)	465 (117)	< 0·0004

*Student's *t* test.

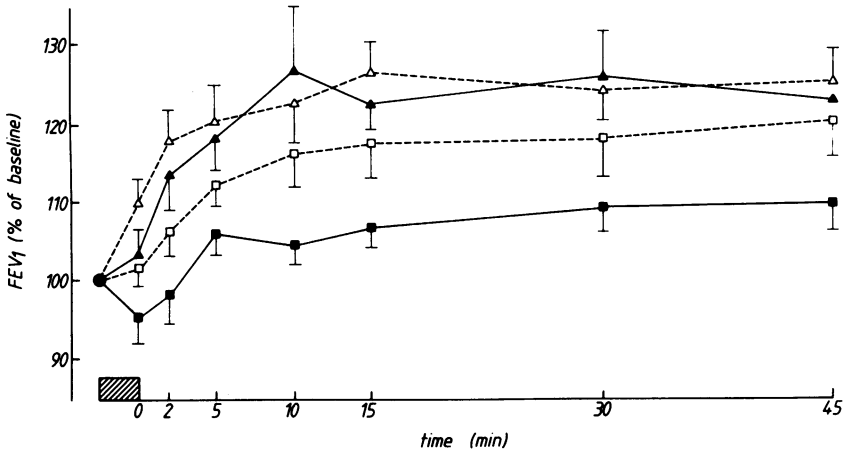

Fig 2 *Effect of Atrovent (squares) and preservative free ipratropium bromide (triangles) in 13 atopic (closed symbols) and 17 non-atopic subjects (open symbols): means with standard errors.*

to Atrovent, maximum bronchodilatation occurring at 30 minutes compared with 45 minutes (fig 1). We have previously shown that both benzalkonium chloride and EDTA may cause bronchoconstriction in patients with asthma, with a response lasting at least 45 minutes. In Atrovent therefore the bronchoconstrictor effects of the preservatives are likely to be competing with the bronchodilator action of ipratropium bromide, in some subjects the initial response being sufficient to cause bronchoconstriction.

A further insight into the possible mechanism or mechanisms by which benzalkonium chloride and EDTA may cause bronchoconstriction comes from observing the influence of atopy on the airways responses (fig 2). The bronchodilatation observed after inhalation of preservative free ipratropium bromide was similar in the two groups; in response to Atrovent the atopic subjects achieved less bronchodilatation than non-atopic subjects, though the difference was not significant. A fall in FEV_1 of 20% or more was experienced by three of the 13 atopic subjects after Atrovent, but only transiently in two of the 17 non-atopic subjects. This reaction could not be attributed to differences in non-specific bronchial responsiveness between the two groups and is likely to relate to the mechanism by which Atrovent provokes bronchoconstriction.

Table 3 *Comparison of airway responses (means with standard errors in parentheses) to Atrovent in atopic and non-atopic subjects*

	Atopic (n = 13)	*Non-atopic (n = 17)*	*p**
Highest FEV_1 after nebulisation (% change from baseline)	14·0 (2·9)	22·0 (4·3)	0·13
Lowest FEV_1 after nebulisation (% change from baseline)	−8·3 (2·8)	0·2 (3·0)	<0·05
Area under the FEV_1-time course curve	311 (121)	759 (177)	<0·05

*Student's *t* test

Benzalkonium chloride is composed of quaternary ammonium compounds and widely used as a bacteriocidal agent. Its molecular conformation contains both hydrophobic and cationic groups, which confer surface active properties thought to be responsible for its bacteriocidal activity.[10] At concentrations above 3 μg/ml it has been shown to stimulate histamine release in vitro from rat peritoneal mast cells.[11] Since it also causes histamine release from mast cells that have been "heat inactivated" (and hence are unresponsive to specific secretagogues such as 48/80), this is thought to be a non-specific effect related to its surface active properties. At concentrations above 1 μg/ml benzalkonium chloride enhances IgE dependent release of the preformed mediator 5-hydroxytryptamine from rodent mast cells.[12] Thus the presence of benzalkonium chloride in Atrovent, with its potential to serve as a mast cell secretagogue, may account for the greater bronchoconstriction observed in patients with atopic asthma, in whom mast cells obtained by bronchoalveolar lavage have been shown to be activated.[13] A similar difference in bronchoconstrictor potency between atopic and non-atopic asthma has been observed after bronchial challenge with adenosine and adenosine 5'-monophosphate (AMP),[14] agonists that also provoke bronchoconstriction by augmenting mediator release from preactivated bronchial mast cells.[15]

Innocenti has documented one patient in whom sensitisation to benzalkonium chloride resulted in more protracted asthma,[16] inhalation of benzalkonium chloride causing immediate bronchoconstriction, which persisted for at least five hours. Although the mechanism was not determined, preinhalation of

sodium cromoglycate completely abolished the bronchoconstriction, suggesting that the immediate airway response may be mediated through a mast cell dependent mechanism. A similar mechanism might have been responsible for a recent case in which nebulised beclomethasone diproprionate containing benzalkonium chloride, in a concentration of 0·2 g/l, appeared to produce bronchoconstriction in asthmatic patients seven months after starting treatment.[17]

Bronchoconstriction by EDTA is thought to occur via a direct contractile effect on airway smooth muscle through calcium chelation.[18] Although the concentration of EDTA required to produce bronchoconstriction under challenge conditions was at least twice that present in Atrovent nebuliser solution, this agent has been shown to increase non-specific bronchial responsiveness in Basenji greyhound dogs.[18] An interaction between benzalkonium chloride and EDTA is therefore possible, with EDTA enhancing airway smooth muscle responsiveness to spasmogenic mediators released from mast cells by benzalkonium chloride.

We conclude that the currently marketed Atrovent nebuliser solution containing preservatives can produce bronchoconstriction in some patients, even when administered in the manufacturers' recommended dose. Bronchoconstriction was not observed when the two preservatives were removed from the solution, and preservative free ipratropium bromide proved to be considerably more effective as a bronchodilator agent than Atrovent. A recent study[19] has reported an inverse relation between the incidence of bacterial contamination of nebuliser solutions and the presence of antibacterial agents in the solution, so removal of these preservatives might result in an increased incidence of bacterial contamination. Contamination would be less likely if the solution were prepared under sterile conditions in unit dose vials.

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References

1 Connolly CK. Adverse reaction to ipratropium bromide. *Br Med J* 1982;**285**:934–5.
2 Jolobe OMP. Adverse reaction to ipratropium bromide. *Br Med J* 1982;**285**:1425–6.
3 Howarth PH. Bronchoconstriction in response to ipratropium bromide. *Br Med J* 1983;**286**:1825–6.
4 Mann JS, Howarth PH, Holgate ST. Bronchoconstriction induced by ipratropium bromide in asthma: relation to hypertonicity. *Br Med J* 1984;**289**:469.
5 Patel KR, Tullet WM. Bronchoconstriction in response to ipratropium bromide. *Br Med J* 1983;**286**:1318.
6 Rafferty P, Beasley R, Howarth PH, Mann JS, Holgate ST. Bronchoconstriction induced by nebulised ipratropium bromide: relation to the bromide ion. *Br Med J* 1986;**293**:1538–9.
7 Beasley R, Rafferty P, Holgate ST. Bronchoconstrictor properties of preservatives in ipratropium bromide (Atrovent) nebuliser solution. *Br Med J* 1987;**294**: 1197–8.
8 Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, Sheffer AL, Spector SL, Townley RG. Standardisation of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;**56**:323–7.
9 Joiner BL, Ryan BF, Ryan TA. *Minitab handbook*. Boston, Mass: PSW Publishers, Duxbury Press, 1985.
10 Harvey SC. Antiseptics and disinfectants: fungicides, ectoparasiticides. In: Goodman LS, Gilman A, eds. *The pharmacological basis of therapeutics*. New York: MacMillan, 1981:978–9.
11 Read GW, Kiefer EF. Benzalkonium chloride: selective inhibitor of histamine release induced by compound 48/80 and other polyamines. *J Pharmacol Exp Ther* 1979;**211**:711–5.
12 Coleman JW, Holgate ST, Church MK, Godfrey RC. Immunoglobulin E decapeptide-induced 5-hydroxytriptamine release from rat peritoneal mast cells. *Biochem J* 1981;**198**:615–9.
13 Tamaika M, Ida S, Yirke S, Ishizaka T, Takishima T. Mast cells in bronchoalveolar lumen of patients with bronchial asthma. *Am Rev Respir Dis* 1984;**129**:1000–5.
14 Phillips G, Rafferty P, Beasley R, Holgate ST. The contribution of histamine release to AMP induced bronchoconstriction in non-allergic asthma [abstract]. *Thorax* 1987;**42**:218.
15 Rafferty P, Beasley R, Holgate ST. The contribution of histamine to bronchoconstriction produced by inhaled allergen and adenosine 5'monophosphate in asthmatics. *Am Rev Respir Dis* 1987;**136**:369–73.
16 Innocenti A. Asma professionale da benzalconio cloruro. *Med Lav* 1978;**69**:713–5.
17 Clark RJ. Exacerbation of asthma after nebulised beclomethasone diproprionate. *Lancet* 1986;ii:574–5.
18 Downes H, Hirshman CA. Calcium chelators increase airway responsiveness. *J Appl Physiol* 1985;**59**:92–5.
19 Barnes KB, Clifford R, Murphy D, Comber P, Bell E, Holgate ST. Bacterial contamination of home nebulisers. *Br Med J* (in press).