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A non-bronchoconstrictor, bacteriostatic preservative for nebuliser solutions

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We have studied the bacteriostatic and airways effects of the preservatives chlorocresol and chlorbutol, to assess if they may be safely used in nebuliser solutions. The bacteriostatic study was carried out according to standard techniques, and the preservatives were able to inhibit the growth of a range of bacteria and yeasts for a period of 28 days. The airways effects were studied in eight asthmatic subjects, who were challenged with either the preservatives or saline (as placebo). Pulmonary function was followed as FEV_1 for 60 min after inhalation, and there was no change in FEV_1 following inhalation. We conclude that these preservatives may be used safely in nebuliser solutions.

Keywords preservatives nebuliser solutions airways effects

Introduction

Nebulisation is a common method of delivering agents to the bronchial tree for the treatment of asthma and related diseases. A large number of bronchoactive drugs are now formulated as solutions for nebulisation. There has been a growing awareness that some of the non-drug additives in nebuliser solutions may cause bronchoconstriction that may be clinically harmful or diminish the effectiveness of the drug constituents (Beasley *et al.*, 1988). The preservatives benzalkonium chloride and ethylene diamine tetraacetic acid (EDTA) were included in drug solutions such as ipratropium bromide, salbutamol and sodium cromoglycate to serve as bacteriocidal agents. It has recently been shown that these two agents are potent bronchoconstrictors in asthma and contribute to the paradoxical bronchoconstriction observed with nebulised ipratropium bromide (Beasley *et al.*, 1987). As a consequence, these additives have been removed from nebuliser solutions, but to minimise bacterial contamination many of these drugs have to be packaged as unit dose vials. However, such packaging is expensive and restricts dosing. In this report we describe a bacteriostatic agent which is not bronchoconstrictor in asthmatics and therefore has potential for use in nebuliser solutions.

Methods

The study was divided into two phases to determine (i) an assessment of the airways effects of the preservatives chlorbutol and chlorocresol in patients with asthma, and (ii) the bacteriostatic properties of the preservatives against a range of organisms.

Inhalation study

Eight mild to moderate asthmatic patients (four male), mean (± s.e. mean) age 32.3 ± 5.1 years participated in the study. Six were atopic on the basis of positive skin prick tests to two or more common aeroallergens and all were non-smokers. All were taking inhaled salbutamol, and three were taking inhaled corticosteroids. None was taking oral xanthines or corticosteroids, and all had been stable for 6 weeks prior to the study. All the subjects had hyperresponsive airways with a geometric mean provocative concentration of histamine required to produce a 20% fall in the FEV_1 (PC_{20}) as determined by a modified Chai technique of 0.82 mg ml^{-1} (range 0.08–5.65 mg ml^{-1}) (Chai *et al.*, 1975), and a baseline FEV_1 of 99.4 ± 4.4% of the predicted value. Subjects gave their written informed consent and the study was approved by the Southampton University and Hospitals Ethics Sub-Committee.

The study was conducted in a double-blind manner, using the preservative combination chlorbutol (0.05% w/v), chlorocresol (0.05%), sodium chloride 0.9% and purified water to 100%, and physiological saline as placebo. The solutions were supplied in 2 ml single-dose vials marked A or B (Rybar Laboratories Ltd, Amersham, Bucks). The solutions were delivered to the subject from an Inspiron Mini-neb nebuliser (CR Bard International, Sunderland, UK) attached to a dosimeter and compressed air at 20 psi delivered at 8 l min^{-1} in such a way that 10 μl of solution was delivered with each breath. Under these conditions, the nebulizer produces an aerosol with a mass median aerodynamic diameter of 6.3 μm and a geometric standard deviation of 1.73 (Newman *et al.*, 1986). Subjects were asked to take 10 consecutive breaths of aerosol from FRC to TLC via a

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mouthpiece so that each subject inhaled a total volume of 100 μl.

On arrival, subjects were rested for 15 min, after which three measurements of FEV_1 were made and the highest value recorded. Providing FEV_1 did not vary from the baseline by > 5%, the test solutions were then administered in a randomised fashion as 10 breaths of aerosol. FEV_1 was measured 2, 5, 10, 15, 30, 45 and 60 min after inhalation. For each subject, the percentage change in FEV_1 from post-saline baseline was plotted against time and the area under the FEV_1-time curve (AUC) calculated by trapezoidal integration. The group mean AUCs for each solution were compared by two-factor analysis of variance.

Bacteriostatic study

The ability of the preservative solution to inhibit the growth of microorganisms was studied according to the recommendations of the British Pharmacopoeia (1988). The following organisms were tested: *Escherichia coli* (1.3×10^7 cfu ml^{-1} of sample), *Staphylococcus aureus* (1.5×10^7 cfu ml^{-1}), *Pseudomonas aeruginosa* (1.6×10^7 cfu ml^{-1}), *Aspergillus niger* (7.5×10^5 cfu ml^{-1}), *Candida albicans* (1.3×10^6 cfu ml^{-1}) and *Saccharomyces rouxii* (1.8×10^6 cfu ml^{-1}). Each organism was employed in a separate test, with 1 ml of each test culture being added to 100 ml of preservative solution. Samples were kept at 22° C for the duration of the test. One millilitre of each sample was removed at each time-point and the number of surviving organisms determined by serial dilution and plate counting at 6, 24 and 48 h and at 7 days and onwards by serial dilution and membrane filtration. The media employed were tryptone soya agar for bacteria, which were incubated at 35° C for 72 h; and sabouraud dextrose agar for moulds and yeasts, incubated at 22° C for 5 days. Plates were examined every 24 h.

The media and diluent were prepared according to the British Pharmacopoeia (1988) but included antagonistic agents (0.5% w/v polysorbate 80 and 0.5% w/v lecithin). The ability of the diluent and media to support the growth of viable organisms was demonstrated by the controls. Prior to the start of the test, the aerobic plate count and yeast/mould counts for the preservative solution were, in all cases, found to be less than 1 ml^{-1} using membrane filtration and the above media and incubation conditions.

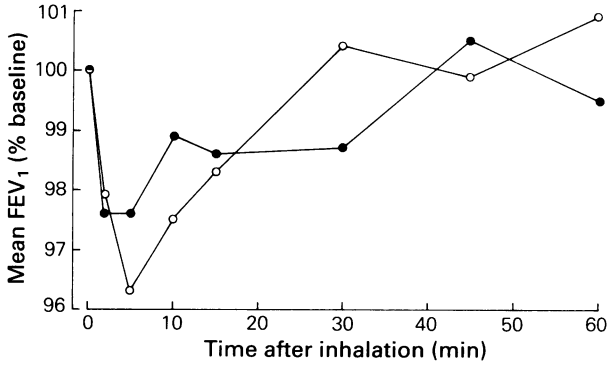

Figure 1 Change in FEV_1 measured as % fall from baseline for 60 min following inhalation of preservative solution (•) and normal saline (○).

Results

Inhalation study

All patients completed the study. There were no adverse reactions to either the preservatives or the placebo. Figure 1 shows the change in the group mean FEV_1 expressed as a percentage of the baseline value over the period of the study. There was little change in pulmonary function after inhaling the test solutions, with the largest fall occurring after saline inhalation, although this was < 4% of the baseline FEV_1. The mean AUC after inhaling saline and preservative was 58.7 ± 9.9 and 58.7 ± 7.5 arbitrary units respectively, which were not significantly different ($P = 0.12$).

Bacteriostatic study

The results of organisms surviving after defined periods of incubation in the preservative medium were the same for all of the test organisms. In all cases, there was a marked reduction in surviving organisms at the time-points tested. Thus, at 6 and 24 h post-incubation, there were < 100 surviving cfu, at 48 h < 10 cfu, and at 7, 14, 21 and 28 days post-incubation, < 1 cfu. These results are well within the guidelines of the British Pharmacopoeia (1988), and confirm the bacteriostatic property of the preservative solution.

Discussion

This study has shown that chlorbutol and chlorocresol are effective bacteriostatic agents and, in the concentrations used do not have adverse airways effects in asthmatic subjects. The dose of the inhaled preservative was chosen because it was similar to that used clinically in 'Rybarvin', a bronchodilator solution containing adrenaline and atropine methonitrate used for more than 40 years which was delivered by hand-held nebuliser; it had never been reported to cause paradoxical bronchoconstriction. To our knowledge, this is the first time that the safety of these agents has been documented in asthmatic subjects, and supports their continued use in commercially available nebuliser solutions. It should be borne in mind, however, that the results of this study will not identify the rare individual who may suffer from an idiosyncratic reaction to the inhaled preservative.

Other preservative agents have been used in formulations intended for nebulisation and have been responsible for inducing bronchoconstriction in asthmatics. This was first seen with inhaled isoprenaline, which contained the preservative sodium metabisulphite (Reisman, 1970). This substance can release SO_2, which is now known to be a potent bronchoconstrictor in some asthmatics and on occasions in non-asthmatics (Sheppard *et al.*, 1981).

This, and other preservatives have now been withdrawn from general use in most formulations, so that for example, ipratropium bromide is now marketed as an isotonic preservative-free solution, the bronchoconstrictors benzalkonium chloride and EDTA having been omitted. It has been demonstrated that following this re-formulation, the drug solution is a more effective bronchodilator agent (Rafferty *et al.*, 1988).

We suggest that the safety of other preservatives used in nebuliser solutions should also be tested in this manner to ensure they do not exert adverse effects on the airways.

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