

Nebulisation of corticosteroid suspensions and solutions with a β -2 agonist

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Abstract

The aim of this study was to determine the output of flunisolide and salbutamol nebulised in combination and BDP and salbutamol nebulised in combination from two different nebulisers under simulated breathing conditions. The BimboNeb and Nebula nebulisers were used to nebulise 3.0ml of two drug mixtures (beclomethasone dipropionate (800 μ g) with salbutamol (5000 μ g) and a mixture of flunisolide (600 μ g) and salbutamol (5000 μ g)). Particle size was determined by inertial impaction. Total outputs of all drugs from both nebulisers were measured using a sinus flow pump under simulated paediatric and adult breathing patterns. The mass median aerodynamic diameters of beclomethasone dipropionate particles from the mixture using the BimboNeb and Nebula were 6.34 and 5.34 μ m, respectively. For salbutamol in this mixture, these values were 3.93 and 3.32 μ m. The mass median aerodynamic diameters of flunisolide particles from the BimboNeb and Nebula were 3.74 and 3.65 μ m, while for salbutamol they were 3.79 and 3.74 μ m, respectively. With the simulated adult breathing pattern, all drug outputs from both mixtures were greater from the BimboNeb than the Nebula after 5 and 10 min nebulisation. With the paediatric breathing pattern and BimboNeb, outputs were lower than when compared to the adult pattern. For the Nebula, outputs were generally similar at 5 and 10min irrespective of the breathing pattern. Drug delivery from the BimboNeb, but not the Nebula, was affected by the simulated breathing pattern. In the majority of cases, nebulising for 10min produced significantly greater drug outputs compared to those after 5min. These results highlight the need to assess amount of aerosolised drug available when drugs are combined, when different nebulisers are used and when they are used with patients of different ages.

Introduction

While the addition of an inhaled long acting β -2 agonist to inhaled corticosteroid gives very good control of asthma, in most patients with asthma, it has been argued that combination inhalers (formoterol/budesonide and salmeterol/fluticasone) may optimise the beneficial actions of each of the individual drugs in the airways (Barnes 2002).

Corticosteroids given by nebulisation are still commonly prescribed to patients with asthma. Even though there is no combined preparation of a corticosteroid and long acting bronchodilator available for nebulisation, corticosteroids are often nebulised in combination with a short acting β -2 agonist. Although this practice was developed to reduce nebulisation time, recent data supporting the benefits of combining β -2 agonists and steroids are likely to increase such usage. It is of concern, however, that data on the effect of mixing and then nebulising a β -2 agonist and a corticosteroid suspension or solution, on the amount of each drug released by the nebuliser is not available. Such data is important as the nominal dose of a drug placed in a nebuliser often bears little resemblance to the dose emitted, which can vary considerably depending on the nebuliser used and breathing pattern of the patient (Barry & O'Callaghan 1998a). The choice of a corticosteroid suspension or solution may also significantly affect the drug delivered (O'Callaghan et al 2000; O'Callaghan et al 2002). When assessing drug delivery from nebulisers it is also essential to take into account the breathing pattern of the patient as this may significantly affect the dose of drug they receive (Collis et al 1990).

The aim of this study was therefore, to estimate the amount of drug contained in particles likely to reach the lungs and the total amount of flunisolide and salbutamol combined and BDP and salbutamol combined that would be inhaled by children and adults from two commonly used nebulisers. Flunisolide was chosen as an example of a steroid in solution and BDP as a steroid suspension. We also describe the HPLC methods developed to allow assay of corticosteroid and

salbutamol concentrations from the same mixture that allowed us to determine their output from the nebulisers studied.

Methods

Nebulisers and medication

The nebulisers and compressors used were the BimboNeb (Mefar, Bovezzo (BS) Italy) and the Nebula (Markos, Monza (MI) Italy). The salbutamol/BDP mixture was prepared by adding 1ml of salbutamol (5000 μ g) (Bronchovaleas, Valeas s.p.a. Pharmaceuticals, Milan, Italy) to the contents of a 2ml respule of BDP (800 μ g) suspension (Clenil A Chiesi, Parma, Italy). The salbutamol/flunisolide mixture was made by adding 1ml of salbutamol (5000 μ g) solution and 0.6ml (600 μ g) of flunisolide solution (Lunibron A, Valeas s.p.a. Pharmaceuticals, Milan, Italy) to 1.4ml of 0.9% saline. Both mixtures had a final volume fill of 3ml.

The compositions of the drug solutions used in this study were as follows:

Bronchovaleas salbutamol (5mg/mL) consisted of salbutamol, methyl p-hydroxybenzoate, ethyl p-hydroxybenzoate and water

BDP suspension was composed of BDP (0.4mg/mL); sodium dihydrogen phosphate dihydrate, sodium chloride, potassium hydrogen phosphate, benzyl alcohol, methyl p-hydroxybenzoate, cetostearyl alcohol, polysorbitan 20, sorbitan monolaurate, propyl p-hydroxybenzoate and pure water.

Lunibron (100mL) contained flunisolide (100mg), propylene glycol (50mL), sodium chloride (450mg) and water (50mL).

Particle-size measurements

The particle-size distributions of both drugs in the aerosol clouds were measured using a glass multi-stage impinger (MSLI, 4 stage) as previously described (O'Callaghan et al 2000). Nebulisers were charged with 3ml of mixture and operated for 5min. The nebulisers and each stage of the MSLI were washed with an appropriate solvent to recover both drugs from the mixture. Both drugs

were quantified simultaneously using fully validated high-performance liquid chromatography (HPLC) methods (see below). Each nebuliser/drug mixture combination was tested 4 times.

Breathing simulation

Total drug output from the nebulisers was measured under simulated breathing conditions as described previously (O'Callaghan et al 2000). Nebuliser drug outputs were deposited on an inspiratory filter (an electrostatic pad in a low dead volume holder) positioned between the breathing simulator and the nebuliser. As in previous studies, drug output was measured using 2 different breathing patterns, paediatric and adult. The tidal volume, respiratory rate and inspiratory fraction were 150ml, 20 breaths/min, 40% and 600ml, 12 breaths/min, 40% respectively. Waste aerosol, released during simulated expiration, was collected on an expiratory filter. Nebulisers were charged with 3ml of mixture and operated for 5min, with the nebuliser mouthpiece connected to the filter assembly. After 5min, nebulisation was interrupted and both filters were changed. Nebulisation was re-commenced for a further 5min (total 10min). Drugs from the mixture remaining in the nebuliser and deposited on the 5 and 10min filters were recovered by dissolution into an appropriate solvent and quantified by HPLC. The method of drug recovery from the filters was validated and found to be >95% in all cases. Each nebuliser/drug mixture combination was tested 4 times with both breathing patterns.

HPLC Methods

New HPLC methods were developed to allow simultaneous determination of both drugs from either mixture during a single chromatography run using an internal standard method. The HPLC column was a Spherisorb ODS1, 5 μ particle size, 100mm x 4.6mm (Waters Ltd, Herts, UK), for both methods. To assay salbutamol and BDP, the mobile phase consisted of Methanol: 0.2% ammonium acetate (68:32) at a flow rate of 1.7ml/min and a column temperature of 40°C using testosterone propionate as the internal standard. For the determination of salbutamol and

flunisolide, the mobile phase was Methanol: 0.1% ammonium acetate (71:29) at a flow rate of 2ml/min and a column temperature of 30°C with benzyl biphenyl as the internal standard. The UV detection wavelength was 239nm for both methods.

Statistical methods

The total mass of each drug deposited in the MSLI and the percentage in each of its four stages were calculated. The 50% cut-off diameter for each stage was known and the cumulative percentages of each drug less than this diameter were calculated.

A log probability plot of aerodynamic size against cumulative percentage of drug undersize was constructed for each drug and from these the MMADs and geometric standard deviations (GSD) were derived. The percentage of particles <4.3 μ m and <6.8 μ m were calculated as the total drug in stage 4 plus the filter and stages 3, 4 plus filter respectively. Mass outputs from the nebulisers under simulated breathing conditions were calculated as % nominal dose for each drug for comparative purposes.

Results are expressed as a mean of 4 experiments, with standard deviation (SD) and 95% confidence interval (CI). Particle size data were compared using a Mann Whitney U test. Mass drug outputs for the different drug/nebuliser combinations and breath patterns were compared using a Two-way ANOVA with Bonferroni post-tests.

Results

Tables 1 and 2 summarise the particle size characteristics of the drug clouds produced when the salbutamol/BDP and salbutamol/flunisolide mixtures were nebulised under constant flow conditions using the BimboNeb and Nebula. When salbutamol and BDP were nebulised together, using either nebuliser, the MMAD was significantly ($p < 0.05$ for BimboNeb; $p < 0.01$ for Nebula) smaller for salbutamol than BDP.

With the same drug combination, the % of drug released in small particles ($< 4.3 \mu\text{m}$) was significantly ($p < 0.05$ for BimboNeb; $p < 0.01$ for Nebula) greater for salbutamol than BDP.

The MMADs calculated for both drugs were larger ($p < 0.05$) following nebulisation by the BimboNeb as was the total drug output of both salbutamol and BDP.

However, when flunisolide was nebulised with salbutamol, a similar proportion of each drug in the cloud was in small particles ($< 4.3 \mu\text{m}$) and the MMADs calculated for salbutamol and flunisolide were almost identical with both nebulisers. The total drug output of both salbutamol and flunisolide was also similar. In all cases, drug recovery from the MSLI and nebuliser was over 94%.

The total amounts of the drugs (expressed as % nominal dose) delivered to a filter placed between the nebuliser and breathing simulator, representing the total amount of drug likely to be inhaled by a patient with the same breathing patterns, are shown in figs 1 and 2.

Using the adult breathing pattern, the outputs of BDP and salbutamol from the mixture were higher from the BimboNeb than the Nebula after 5 minutes ($p < 0.01$) and 10 minutes of ($p < 0.001$) nebulisation.

The effect of breathing pattern on drug output was dependent on the nebuliser used. Using the adult breathing pattern, the BimboNeb delivered 181 μ g (29.4) of BDP and 1406 μ g (105) of salbutamol to the filter in 10minutes, slightly more than the 132 μ g (22.4) of BDP but significantly ($p<0.01$) more than the 1083 μ g (84.4) of salbutamol delivered using the paediatric pattern. However, the outputs of BDP and salbutamol from the Nebula were similar after 5min and 10min, whichever breathing pattern was used.

Using the adult breathing pattern and the salbutamol/flunisolide mixture, the outputs of both drugs were significantly higher from the BimboNeb than the Nebula after 5min ($p<0.01$) and 10 min ($p<0.001$) nebulisation. Using the paediatric breathing pattern, both nebulisers delivered a similar amount of flunisolide and salbutamol to the filter in 5min, but after 10min the BimboNeb delivered significantly more ($p<0.01$ for flunisolide; $p<0.001$ for salbutamol) of both drugs. Using the adult breathing pattern, the BimboNeb delivered 123 μ g (13.3) of flunisolide and 943 μ g (51.7) of salbutamol to the filter in 10min, slightly more than the 99.5 μ g (4.8) of flunisolide and 802 μ g (36.4) of salbutamol delivered using the paediatric pattern. The Nebula only delivered slightly more flunisolide and salbutamol to a filter in 10min using the paediatric pattern compared to the adult.

Increasing nebulisation time from 5 min to 10 min caused a substantial increase in the outputs of both drugs from the salbutamol/BDP mixture, for both nebulisers. For the salbutamol/flunisolide mixture, increasing nebulisation time had less effect on output, particularly for the Nebula.

Discussion

Inhaled steroids play an extremely important part in the treatment of patients with asthma and are used as a first line prophylactic agent for children and adults (British Thoracic Society 1997).

Although important side effects are rarely seen in users of low dose inhaled steroids, there are concerns over the potential effects of high dose inhaled steroids (Todd et al 2002a). In 1998, the Committee of Safety in Medicine in the United Kingdom concluded that clinically important systemic adverse effects can occur at licensed doses of inhaled corticosteroids (Committee on Safety of Medicines (CSM) & Medicines and Health Care Products Regulatory Agency (MHRA) 1998). A major problem exists, however, in assessing clinical trials of inhaled steroids and in assessing the dose patients who experience side effects. The problem is that the dose the patients are likely to have inhaled is rarely evaluated which is of concern as it may bear little resemblance to the prescribed 'nominal' dose. Major differences in the dose inhaled may occur between different drug delivery devices (Barry & O'Callaghan 1999). We have previously shown that the dose of budesonide a 10 year old patient is likely to inhale from a breath enhanced open vent nebuliser is 4 times that available from an open vent device and twice that available from a conventional nebuliser (O'Callaghan & Barry 1999). However, patients and parents buying a nebuliser and those prescribing the drugs are usually unaware that the dose of drug inhaled may vary by a factor of four depending on their choice of nebuliser.

In this paper, we have examined the effect of nebulising inhaled corticosteroids mixed with the β -2 agonist salbutamol. While such clinical practice is not uncommon, the effect on drug output of steroid or β -2 agonist has not previously been reported. To allow us to determine drug output from this combination, we developed HPLC methods that allowed the aerosol collected to be assayed simultaneously for the corticosteroid and for salbutamol. The study was divided into two parts. First was the estimation of the total amount of β -2 agonist and corticosteroid leaving the nebulisers and the amount of each drug contained in various particle size fractions. As the breathing pattern of patients may have a profound effect on the dose they are likely to inhale from a nebuliser, the

effect of an adult and paediatric breathing pattern on the total dose of the drugs likely to be inhaled was also determined.

Corticosteroid preparations for nebulisation are usually formulated as suspensions. The exception to this is flunisolide which is available as a solution. The nebulisation of suspensions is significantly different from solutions as when nebulised a corticosteroid particle is surrounded by an envelope of carrier fluid. This may result in fewer corticosteroid particles being able to escape from the nebuliser due to their 'enhanced' size with those particles that do escape tending to be relatively large. Indeed, early attempts to nebulise a 50µg/ml suspension of BDP resulted in little drug being released in particles small enough to enter the lung (O'Callaghan 1990) and a poor clinical response (Storr et al 1986; Webb et al 1986). One possible way to increase the amount of BDP likely to be delivered is to increase the drug concentration. The more concentrated suspension of BDP that is commercially available was therefore used in this study. Increasing the concentration, however, may also have an effect on particle-size, although this was not determined in this study. Previous work had shown that the nebulisation of 2ml of a formulation containing 400µg/ml of BDP resulted in an aerosol cloud with a MMAD of 6.4µm for the Bimboneb and 5.4µm for the Nebula.

When BDP and salbutamol were nebulised as a mixture, the % of each drug in small droplets (<4.3µm) was much higher for salbutamol than for BDP and was also affected by the choice of nebuliser. This difference may be explained by the fact that when BDP particles are nebulised they are only contained in a few of the aerosol particles leaving the nebuliser. The particles of BDP are surrounded by a fluid envelope that is likely to increase the droplet size further. The droplets not containing BDP are likely to be more numerous and smaller and are made up of the 'carrier' solution of salbutamol.

Another way of improving the delivery of nebulised corticosteroids to the lung is to improve the solubility of a drug by adding a co-solvent and/or other additives such as surfactants or buffers

(O'Callaghan & Barry 1997). Drug output and the percentage of drug contained in small particles are normally greater from a solution than a suspension. The flunisolide formulation (Lunibron A, Valeas s.p.a. Pharmaceuticals, Milan, Italy) used in this and our previous study (O'Callaghan et al 2002) contained propylene glycol, which acted as a co-solvent to promote the dissolution of the flunisolide. The particle size characteristics of the flunisolide cloud from the BimboNeb and Nebula were almost identical, with an MMAD of 3.9 μm and approximately 60% of the drug released in particles <4.3 μm . Only 19% of the drug was contained in particles >6.8 μm , which suggests that the oropharyngeal deposition may be lower than for BDP. As expected when nebulising the combined solution of salbutamol and flunisolide there was no difference in particle size for the two drugs.

The outputs of BDP and flunisolide from the BimboNeb and Nebula have been measured previously using the same methodology as the present study (O'Callaghan et al 2000; O'Callaghan et al 2002). Although the results are not directly comparable due to the combination of salbutamol with the corticosteroids and the larger volume fills, the MMADs of the steroid particles were very similar (O'Callaghan et al 2000; O'Callaghan et al 2002). This suggests there is little effect of combining the steroids with salbutamol on particle size of the steroid aerosol produced.

It is known that the lung deposition of inhaled medications is affected by patient factors and also the delivery device. The dose of medication that reaches the airways may be affected by nebuliser type, fill volume, and driving gas flow (Hess et al 1996), breathing pattern (Barry & O'Callaghan 1998b) and even the distance of the nebuliser from the face when a facemask is used (Everard et al 1992). In this study, we evaluated the effect of either a simulated adult or paediatric breathing pattern on the dose captured on a filter between the nebuliser and the breathing simulator machine. For both nebulisers and both the adult and paediatric breathing patterns, slightly more salbutamol was delivered to the breathing simulator filter in 5 min when mixed with BDP compared to with flunisolide. Extending the nebulisation time to 10 min significantly increased the output of

salbutamol to the filter in the majority of cases. This increase was less significant with the Nebula for both breathing patterns when salbutamol was co-nebulised with flunisolide. As can be seen from the results section, the drug output from the two nebulisers could not be predicted and varied with each device and also with the breathing pattern used.

Conclusion

The results of this study suggest that salbutamol may be nebulised with corticosteroid suspensions and solutions. However, unless the combination of drugs and nebulisers are tested in association with the breathing pattern of the patients likely to use the nebuliser, it is impossible to estimate the dose the patient is actually likely to inhale from the prescribed, nominal, dose. We argue that detailed information should be made available to clinicians on the dose of drug that is likely to be inhaled by their patient for each nebuliser drug combination. Failure to do this is to accept that children and adults with asthma may receive significant differences in the dose of steroid or β -2 agonist without anyone being aware.

Legends:

Figure 1: Breathing simulation data for salbutamol/BDP mixture. Amount of each drug deposited on a filter shown as % nominal dose, each bar represents the mean of 4 measurements. Grey and white bars represent drug deposited after 5min and 10min nebulisation respectively.

Figure 2: Breathing simulation data for salbutamol/flunisolide mixture. Amount of each drug deposited on a filter shown as % nominal dose, each bar represents the mean of 4 measurements. Grey and white bars represent drug deposited after 5min and 10min nebulisation respectively.

Table 1 Particle-sizing data for Salbutamol/BDP mixture from BimboNeb and Nebula nebulisers

	BimboNeb		Nebula	
	Salbutamol	BDP	Salbutamol	BDP
MMAD (μm)	3.93 (0.22)	6.34 (0.27)	3.32 (0.19)	5.34 (0.40)
GSD	2.43 (0.09)	1.80 (0.05)	2.54 (0.05)	1.95 (0.06)
Total weight of drug in cloud (μg)	2697 (189)	289 (31.1)	1863 (124)	172 (24.9)
% of total amount of drug leaving nebuliser in particles <4.3 μm	51.1 (2.8)	29.1 (3.6)	58.0 (2.5)	38.5 (4.2)
% of total amount of drug leaving nebuliser in particles <6.8 μm	68.6 (2.3)	51.4 (2.0)	76.4 (1.9)	63.8 (4.1)

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation;
mean (n=4) and s.d. in parentheses are shown.

Table 2. Particle-sizing data for Salbutamol/Flunisolide mixture from BimboNeb and Nebula nebulisers

	BimboNeb		Nebula	
	Salbutamol	Flunisolide	Salbutamol	Flunisolide
MMAD (μm)	3.79 (0.25)	3.74 (0.24)	3.74 (0.18)	3.65 (0.22)
GSD	2.24 (0.08)	2.28 (0.10)	2.21 (0.04)	2.26 (0.02)
Total weight of drug in cloud (μg)	1918 (364)	243 (44.1)	2087 (68)	264 (11.2)
% of total amount of drug leaving nebuliser in particles <4.3 μm	50.3 (4.6)	51.7 (4.6)	48.5 (2.7)	50.2 (3.2)
% of total amount of drug leaving nebuliser in particles <6.8 μm	74.1 (3.6)	74.74 (3.3)	72.5 (2.4)	73.7 (2.7)

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation;
mean (n=4) and s.d. in parentheses are shown.

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Figure 1

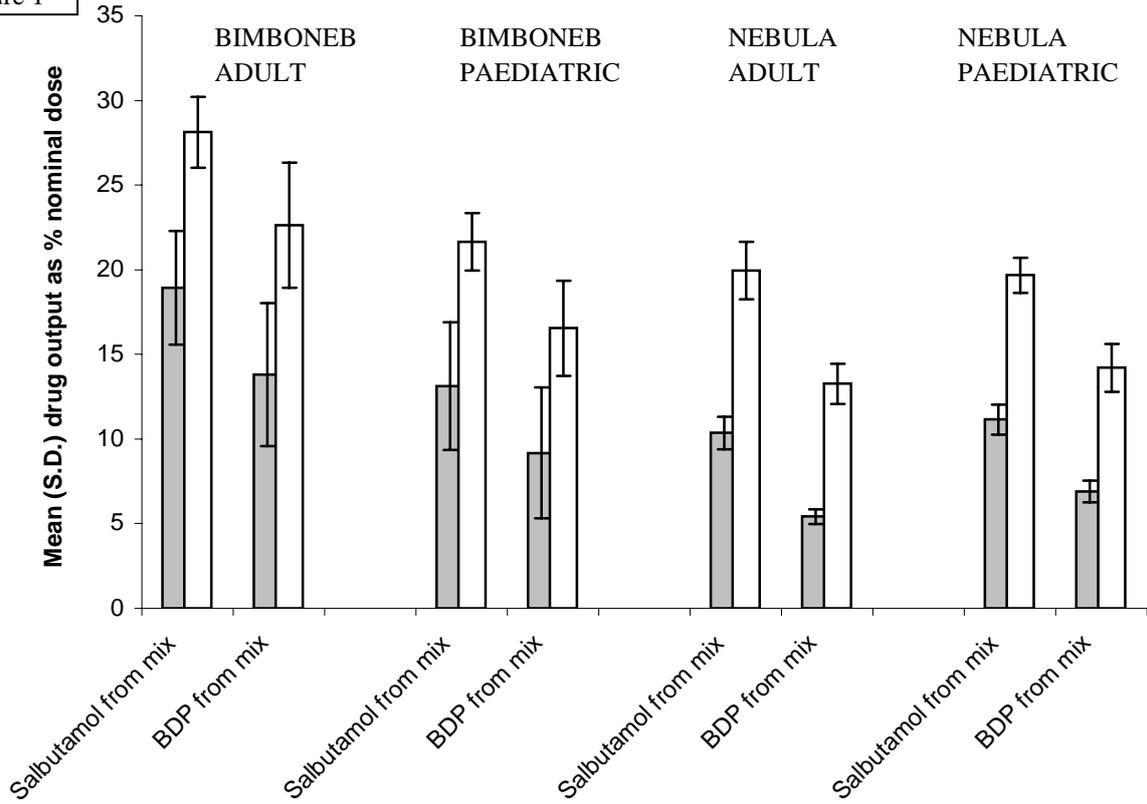


Figure 2

