

An in vitro analysis of the output of budesonide from different nebulizers

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Background: Inhaled corticosteroids are increasingly used in the treatment of asthma, and many different nebulizers are available to aerosolize steroid medications. There are few comparative data on their ability to do so.

Objective: Our purpose was to determine the particle size and mass output of budesonide nebulizer suspension from different nebulizers.

Methods: In vitro measurement of drug particle size and total drug output from 3 nebulizers (the Pari LC Plus, the Pari LC Star, and the Medicaid Ventstream) was performed under simulated breathing conditions. Nebulizers were charged with 2 mL (500 µg) of budesonide suspension. A sinus pump was used to draw aerosol from the nebulizers onto a filter during simulated inspiration at tidal volumes of 150 and 600 mL, mimicking pediatric and adult use. Aerosol particle size was determined separately by inertial impaction.

Results: The LC Plus nebulizer had the highest initial output rate and delivered the most budesonide at both breathing patterns. The maximal output rates of the Ventstream and LC Star nebulizers was half that of the LC Plus, but the LC Star nebulizer continued nebulization for longer and delivered twice as much budesonide as the Ventstream did. However, the Ventstream produced the smallest particles, mass median diameter 3.1 µm compared with 3.8 µm for the LC Star and 4.1 µm for the LC Plus.

Conclusions: This study has identified differences among the nebulizers that would not have been apparent with current standards for nebulizer assessment. Incorporation of breathing simulation in the study imitates patient use and allows effective nebulization times to be predicted. The results suggest that the nebulizers studied would deliver different masses of budesonide to the lungs and to the upper airway. This may have important consequences in determining the efficacy and side effect profile of budesonide. (*J Allergy Clin Immunol* 1999;104:1168-73.)

Key words: Nebulizers and vaporizers, budesonide, drug administration—inhalation, asthma

Although nebulizers are commonly used in the treatment of asthma and chronic obstructive airway disease, physicians are often uncertain of the amount of drug placed in the nebulizer that reaches the patients' lungs. With many nebulizers now available, differences among

Abbreviations used

GSD: Geometric SD
MMAD: Mass median aerodynamic diameter
MSLI: Multistage liquid impinger

them may affect disease control. Patients and physicians need clear information about their inhalational drug delivery device to optimize therapy.

Nebulizers are claimed to be inefficient drug delivery devices, with much of the drug placed in the nebulizer never reaching the patient.¹ Conventional jet nebulizers produce aerosol throughout the respiratory cycle, so that at least half the nebulizer output is produced during exhalation and is wasted.² Recent improvements in nebulizer design have been made to increase the output of nebulizers and to reduce expiratory losses.³ "Breath-enhanced, open-vent" nebulizers, such as the Ventstream (Medicaid, Pagham, UK) and the Pari LC Plus (Pari GmbH, Starnberg, Germany) draw additional air into the nebulizer, increasing output. A valve system controls the airflow through the nebulizer, closing during expiration. Increased nebulizer output therefore occurs only during inspiration, and a smaller proportion of the output is wasted. This type of nebulizer has been shown to double the delivery of salbutamol to the lung compared with a conventional nebulizer.⁴

However, it is known that different nebulizers are not equally effective in delivering different drug formulations.⁵ Furthermore, the output of breath-enhanced, open-vent nebulizers is affected by the patients' inspiratory flow,⁶ and they may have different output characteristics when used with children or adults with low inspiratory flows.

The aim of this study was to determine the particle size and mass output of a corticosteroid, budesonide, from a number of different breath-enhanced, open-vent nebulisers currently used in our clinical practice to provide such information.

METHODS

Nebulizers and medication

Three jet nebulizer-compressor combinations were assessed: the LC Plus nebulizer and Turboboy compressor (38G00, operating pressure 0.7 bar, driving gas flow 5 L/min (Pari GmbH, Starnberg, Germany), the LC Star nebulizer and Turboboy compressor (38G00, Pari GmbH), and the Ventstream nebulizer and Portaneb compressor (operating pressure 0.95 bar, driving gas flow 6 L/min, Medicaid, Pagham, UK) were assessed with budesonide nebulizer suspension (500 µg in 2 mL, Pulmicort, Astra, Kings Langley, UK).

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These nebulizer-compressor combinations are marketed for use together and are used in our clinical practice. They are all examples of breath-enhanced, open-vent nebulizers, which have been recommended for use with corticosteroids. Clinicians may assume that these 3 nebulizers, of the same type, are equivalent. Six nebulizers of each type were used. Each was washed 10 times according to the manufacturers' instructions before first use.

Particle size measurements

Particle size distribution of the nebulizer output was measured with use of a glass multistage liquid impinger (MSLI).⁷ This draws aerosol through a series of stages, each connected by progressively smaller jets. Aerosol velocity therefore increases through each jet, resulting in the deposition of smaller particles at each stage. Flow through the MSLI and nebulizer was constant at 30 L/min.

The nebulizers were charged with 2 mL (500 µg) of budesonide suspension and connected to the MSLI inlet by a short plastic sock. Ten milliliters of ethanol was placed in each stage of the MSLI to dissolve any impacted budesonide and prevent re-entrainment of drug particles. The MSLI and nebulizer were switched on and operated for 5 minutes.

After each experiment the stages were washed with ethanol, and the amount of drug collected in each stage was assayed by HPLC, described below. Each nebulizer-compressor combination was tested 4 times.

The size of drug particles depositing on each stage of the MSLI, and hence the size distribution of the aerosol cloud, was determined by calibrating the MSLI with an aerosol of known particle size distribution. The cut-off diameters for stages 1 to 4 of the MSLI were 14.3, 9.6, 6.1, and 1.4 µm respectively.

Breathing simulation

Total drug output from the nebulizers was also measured with use of a breathing simulator, the Pari Sinus Breathing Simulator (Pari GmbH), which allows the simulated tidal volume, respiratory rate, and inspiratory time to be independently adjusted.⁸ The experimental apparatus is illustrated in Fig 1. Two milliliters (500 µg) of budesonide nebulizer suspension were added to the nebulizer, which was attached to the breathing simulator. Electrostatic filter pads were used to collect the aerosolized drug, held in a plastic filter assembly (dead space 11 mL). Nebulizers were connected to the filter assembly by the T-piece or mouthpiece supplied by the nebulizer manufacturer. Waste aerosol released during "expiration" was collected on the expiratory filter. Nebulizers were operated for up to 15 minutes, and nebulization was interrupted briefly after each minute up to 5 minutes and again after 10 and 15 minutes, to allow the inspiratory filter to be changed. In this way the drug output at different times could be determined. The process of changing the filter took less than 10 seconds. In a separate series of experiments nebulization continued for 5 minutes and the amount of drug collected on the filter was compared with that from the main experiments.

Two different breathing patterns were used, one to represent a child and noted hereafter as the "pediatric" breathing pattern, and one to represent an adult, noted hereafter as the "adult" breathing pattern. The tidal volume, respiratory rate, and inspiratory time were 150 mL, 20 breaths/min, and 40% and 600 mL, 12 breaths/min, and 40%, respectively. These settings are representative of published values⁹ and give a minute volume, maximum inspiratory flow, and mean inspiratory flow of 3 L, 11.8 L/min, and 7.5 L/min and 7.2 L, 28.3 L/min, and 18 L/min for the 2 breathing patterns, respectively.

Each nebulizer was assessed at each breathing pattern 6 times up to 5 minutes and 3 times up to 15 minutes. At the end of each experiment the inspiratory filter was removed from the housing and

washed with an appropriate solvent. The amount of drug collected on the filter was determined by HPLC. The recovery of drug from filters primed with a known amount of budesonide was 100.4% (95% confidence intervals 97.5% to 103.3%). The filters collected all drug particles, the recovery of drug from the second of 2 filters in line being less than the limit of detection of the assay.

In pilot studies drug depositing on all parts of the experimental apparatus was measured, allowing the total recovery of drug to be estimated as a percentage of that added. A mean of 88% of the budesonide added was recovered (95% confidence intervals 86.8% to 89.8%). Total drug recovery was not dependent on the nebulizer type. We assume that the "missing" 12% was retained in the nebulizer or lost through the nebulizer's expiratory valves as they closed.

Running the nebulizer continuously for 5 minutes without changing the filter increased the filter deposition by a mean of 16%, consistent with a 10-second interruption of nebulization each minute while the filter was changed.

To determine the passive deposition of drug particles on the inspiratory filter, experiments were repeated with budesonide and the nebulizers attached to the breathing simulator as shown in Fig 1 but with the breathing simulator switched off. Eight experiments were undertaken and a mean (SD) of 8.7 µg (12.0) of budesonide was recovered from the inspiratory filter, representing less than 2% of the budesonide added to the nebulizer.

Drug assays

Budesonide was assayed in our laboratory with use of reversed-phase HPLC with UV detection at a wavelength of 254 nm. A 5-cm Spherisorb ODS1 column (4.6 mm inner diameter) was used with ethanol/water as the mobile phase. The internal standard fluocinolone acetonide was added to the filters or MSLI stages immediately after nebulization. The limit of detection of the assay was 0.1 µg/mL budesonide. The system response was linear over a range of budesonide concentrations from 0.1 to 200 µg/mL. The coefficient of variation of the assay was 2.6% (at a concentration of 1 µg/mL).

Statistical methods

The size distribution of the aerosol cloud was determined from the amount of drug recovered from each stage, the MSLI having been previously calibrated with an aerosol of known particle size distribution. From this a log-probability plot was constructed,¹⁰ and the mass median aerodynamic diameter (MMAD) and the geometric SD (GSD) were determined. Recovery of drug from the breathing simulator filters, and of that contained in particles smaller than 6.1 µm (representing drug collected on stage 4 and the filter of the MSLI), was compared by ANOVA. Analysis was undertaken with use of Minitab statistical software (Clecom, Birmingham, UK).

RESULTS

Particle size

For the nebulizers assessed with the MSLI, the MMAD, the GSD, the mass of drug, and the percentage nebulizer output collected on stage 4 and the filter of the MSLI, representing drug particles smaller than 6.1 µg, are given in Table I.

Under the constant flow used by the MSLI, the LC Star delivers the most drug and deposits the most drug on stages 4 and filter (ANOVA, $P = .016$). The LC Plus nebulizer has the largest particle size output, MMAD 4.1 µm. The Ventstream, with the smallest particle size output (MMAD 3.1 µm, ANOVA, $P = .001$), delivers a smaller mass of budesonide to stage 4 and filter than the LC Star but a significantly higher percentage ($P < .001$),

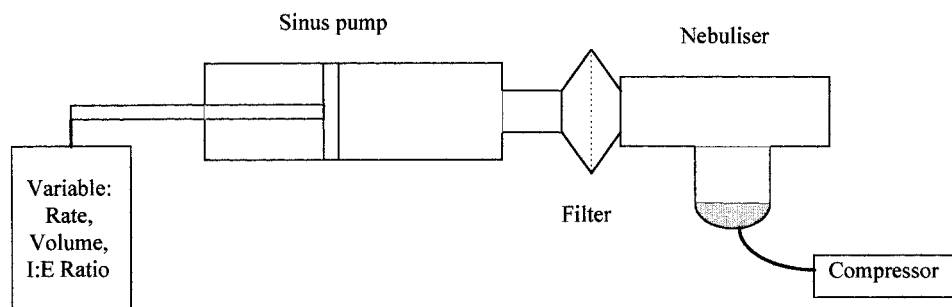


FIG 1. Schematic diagram of breathing simulation apparatus.

TABLE I. Mean and (95% confidence intervals)

	MMAD (μm)	GSD	Drug <6.1 μm (μg)	Percent <6.1 μm
LC Plus/Turboboy	4.1 (3.9-4.2)	2.1 (2.0-2.2)	95.0 (87.0-103.1)	69.8 (67.6-72.0)
LC Star/Turboboy	3.8 (3.5-4.0)	1.9 (1.8-1.9)	118.1 (113.1-123.1)	77.2 (71.3-83.1)
Ventstream/Portaneb	3.1 (2.8-3.3)	2.0 (1.9-2.1)	101.8 (91.8-111.8)	87.0 (85.3-88.6)

For nebulizers assessed with MSLI, the MMAD, GSD, mass of drug, and percentage nebulizer output collected in particles smaller than 6.1 μg are given. ANOVA shows significant differences among nebulizers in MMAD ($P = .016$) and mass and percentage of drug in small particles ($P 0.001$).

delivering less drug in large particles to the upper stages of the impinger.

Breathing simulation

All nebulizers had a constant initial output of drug, which declined after a variable time (Figs 2 and 3). We report the maximal output rate, in micrograms of drug released per minute, the total output over 15 minutes, in micrograms, and the time taken for 90% of the total output to be released (Table II).

Analysis of variance showed a significant difference in drug output among the nebulizers at both breathing patterns ($P < .001$). The LC Plus nebulizer had the highest output of budesonide at both breathing patterns, delivering a mean (SD) of 91.9 μg (3.1) and 110.8 μg (8.8) of budesonide in 15 minutes at the pediatric and adult breathing patterns, respectively. This nebulizer was also the fastest, delivering 90% of the budesonide in 7 and 5 minutes for the 2 breathing patterns. The LC Star nebulizer took longer to deliver 90% of the total output of budesonide, 11.4 and 8.5 minutes for the 2 breathing patterns, and, at the pediatric breathing pattern, delivered less drug than the LC Plus (66.5 μg [5.9] vs 91.9 μg [3.1], $P = .007$). The difference between the 2 nebulizers at the adult breathing pattern (100.8 μg [2.0] vs 110.8 μg [8.8]) was not statistically significant ($P = .2$).

The Ventstream nebulizer had a maximum output rate of budesonide similar to that of the LC Star, but it stopped releasing drug after a much shorter time so that the total output at both breathing patterns (32.3 μg [4.2] and 47.9 μg [11.8], respectively) was only half as much as that of the LC Star ($P = .004$ and $.017$, respectively).

Fig 4 shows the calculated amount of budesonide collected on the filter of the breathing simulator in particles smaller and larger than 6.1 μm aerodynamic diameter,

assuming that the particle size output of the nebulizers is the same as that measured by the MSLI and does not change during the breathing cycle. At the adult breathing pattern, the LC Star nebulizer delivers the same amount of budesonide in small particles as the LC Plus ($P = .9$) but less in larger particles that may have an impact in the upper airway. The Ventstream delivers significantly less drug in both small and large particles than the other nebulizers at the pediatric breathing pattern (vs the LC Plus, $P < .001$, vs the LC Star, $P = .006$) and the adult breathing pattern (vs the LC Plus, $P = .014$, vs the LC Star, $P = .026$).

DISCUSSION

This in vitro study has detected large differences in the delivery of a corticosteroid drug from different nebulizers. Patients using one nebulizer may receive more than twice as much drug from it compared with another. This may be crucial in the delivery of corticosteroids, where dose-dependent effects may occur.¹¹ It is also important to define the dose of medication administered in drug trials and dose equivalence studies. Therapeutic failure may occur in such trials, or the wrong conclusions may be drawn, if patients are categorized on the basis of the dose of drug placed in different nebulizers, with no regard to the amount of drug delivered from them.

The time taken to deliver 90% of the nebulized medication also varied widely among nebulizers. Patient compliance may fall if prolonged nebulization is needed and, from our results, patients can be given a time after which nebulization may be stopped, even if the chamber appears to be producing aerosol. Patients should be given a specific time for nebulization, as has been suggested previously.¹²

Our data are supported by other studies with some of these nebulizers. Berg et al¹³ found that the largest output of budesonide came from the nebulizer producing the

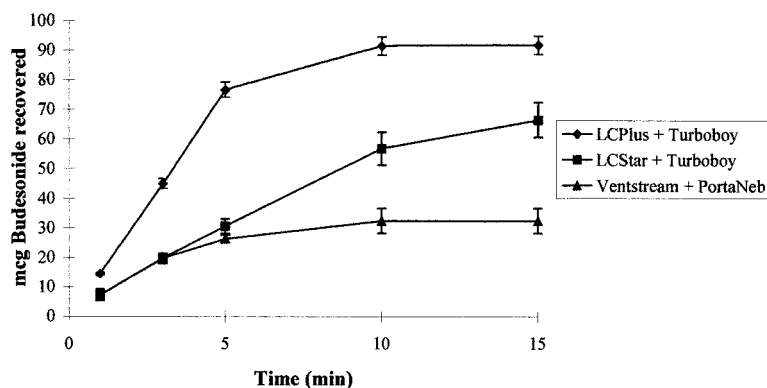


FIG 2. Amount of budesonide collected on filter of breathing simulator over 15 minutes, pediatric breathing pattern. Error bars, 1 SD.

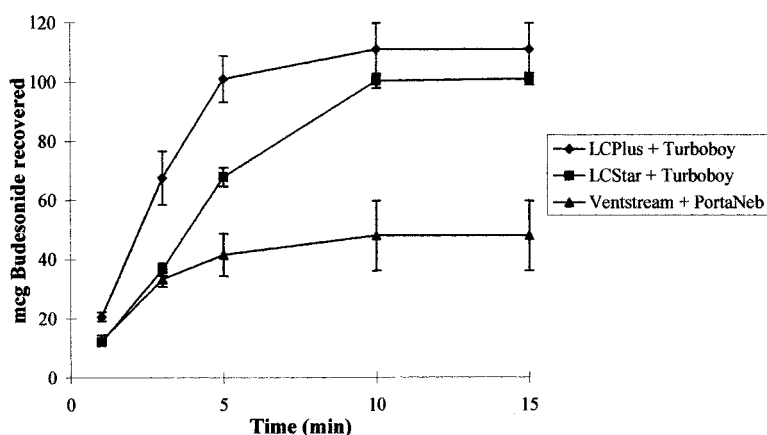


FIG 3. Amount of budesonide collected on filter of breathing simulator over 15 minutes, adult breathing pattern. Error bars, 1 SD.

TABLE II. Maximal output rate, in micrograms of drug released per minute, total output over 15 minutes, in micrograms, and time taken for 90% of total output to be released

Nebulizer	Breathing pattern	
	Pediatric	Adult
First minute output rate ($\mu\text{g}/\text{min}$, mean [SD])		
LC+	14.5 (0.4)	20.7 (1.5)
LCStar/TB	7.4 (0.4)	12.1 (0.8)
Ventstream	7.3 (2.0)	12.6 (1.7)
Total output over 15 minutes (μg , mean [SD])		
LC+	91.9 (3.1)	110.8 (8.8)
LCStar/TB	66.5 (5.9)	100.8 (2.0)
Ventstream	32.3 (4.2)	47.9 (11.8)
Time to 90% output (min [decimal], mean [SD])		
LC+	7.03 (0.2)	4.9 (0.1)
LCStar/TB	11.4 (0.9)	8.5 (0.1)
Ventstream	7.0 (1.3)	5.7 (1.6)

ANOVA shows significant difference in drug output among nebulizers at both breathing patterns ($P < .001$).

largest particles, similar to our data where the Ventstream (MMAD 3.1 μm) released 23.4% of the budesonide added compared with the LC Star nebulizer (MMAD 3.8 μm), which released 30.7%. The difference may be the result of the size of the budesonide crystals in suspension

and the large internal surface area of the Ventstream increasing the "dead volume" of the nebulizer.

With use of a breathing simulator, Nikander and Wunderlich¹⁴ studied the output of budesonide from the LC Plus nebulizer with different compressors. With the Tur-

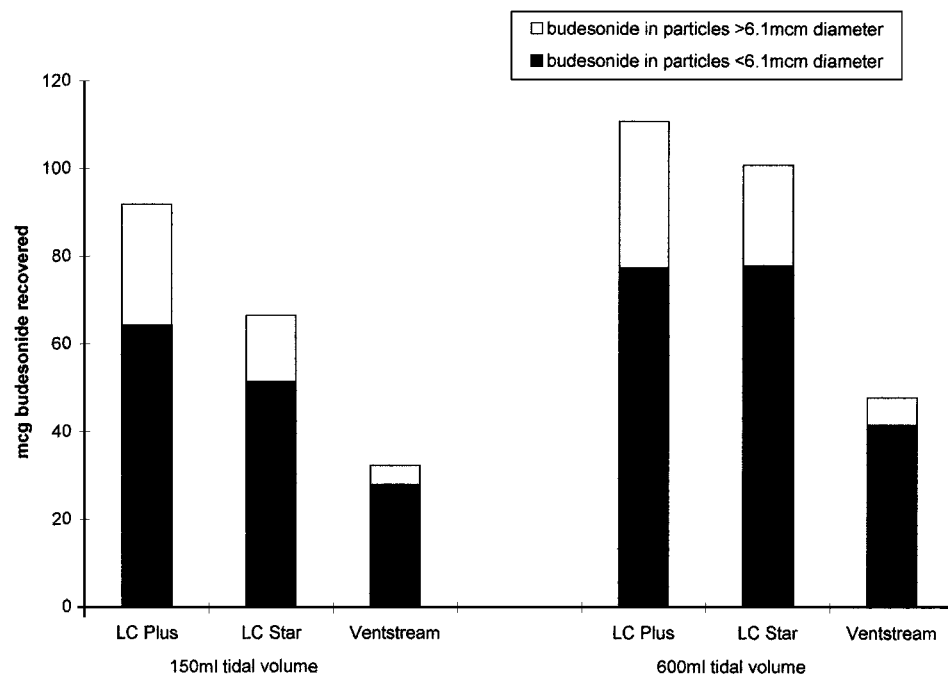


FIG 4. Calculated amount of budesonide collected on filter of breathing simulator in particles smaller than 6.1 μ m and larger than 6.1 μ m.

boboy compressor (driving gas flow 4.3 L/min), 23.2% of the budesonide was recovered on the inspiratory filter, and the MMD was 5.0 μ m, compared with 19.6% and 6.0 μ m with the Walkboy compressor (driving gas flow 2.8 L/min). Thus reducing the driving gas flow increases particle size and has a small effect on budesonide output. In our study with the adult breathing pattern, 22.2% of the dose placed in the LC Plus nebulizer was recovered from the inspiratory filter, remarkably similar to the results of Nikander and Wunderlich.¹⁴

The patient's inspiratory flow may also alter the nebulizer output. Knoch and Wunderlich⁶ measured the output of drug solution from the LC Plus nebulizer at different inspiratory flows. The output increased by a factor of 3 as the additional inspiratory flow increased to 20 L/min and the droplet size decreased by 8%. At flows above 20 L/min the droplet size fell by 22%. We assume that the inspiratory flows used in this study are representative of patients. There are, however, very few data on the breathing pattern of patients with airway obstruction. The breathing simulator parameters used in this study are similar to those obtained in children with use of spacer devices.⁹ Breathing patterns are often irregular¹⁵ and the use of breathing simulators that produce more realistic breathing pattern deserves further study.

A correlation between in vitro particle size measurements and drug delivery has been suggested.^{4,16} However, none of the in vitro methods are perfect for predicting the delivery pattern of all aerosols. For instance, the glass MSLI used in our experiments draws air at a constant flow of 30 L/min through the nebulizer, similar to the peak inspiratory flow of the adult breathing pattern.

Although ambient conditions may alter measured particle size in some inertial impactors,¹⁷ the presence of fluid in the impaction stages of the MSLI increases humidity but still does not replicate conditions in the respiratory tract, where particle growth may occur.¹⁸ Another widely used method, laser diffraction, measures the size of all particles that pass through the laser beam, regardless of whether they contain drug, and is not appropriate for the analysis of nebulized suspensions. The output of nebulizers under constant flow conditions may differ from those obtained with breathing simulation.⁸

There have been few clinical studies of lung deposition of nebulized aerosols in children. Alderson et al¹⁹ found that lung deposition increased with age and that those with normal ventilation scans had uniform deposition of labeled aerosol, whereas those with areas of reduced ventilation had corresponding areas of reduced deposition. However, others^{20,21} have found no relationship between age and total lung deposition of nebulized aerosols, although deposition in infants may be lower than in older children.²² These studies showed wide interindividual variation in lung deposition, and small studies have failed to show a relationship between age²³ or clinical effect²⁴ and filter deposition. The deposition pattern is altered in disease, with more central deposition seen in children with lung damage.²¹ It may be that a device producing smaller particles, such as the Ventstream, will give a greater and more uniform drug delivery to the peripheries of the diseased lung.

Drug collected on filters during the breathing simulation experiments represents all the medication delivered to the patient, not just that delivered to the lungs. Lung deliv-

ery may be estimated by multiplying the filter drug deposition by the fraction of drug below a certain size from the particle sizing experiments. We have assumed that lung deposition is related to aerosol particle size and that the cutoff value used here, 6.1 μm , is appropriate for delineating between lung and extrapulmonary drug delivery. Recent debate has suggested that a smaller particle size may be appropriate for the delivery of drug to the lungs.²⁵

Inhaled medications are increasingly used in the treatment of asthma and other diseases such as croup and bronchopulmonary dysplasia.²⁶ The inhalational route allows drug to be delivered directly to its site of action, and nebulizers are used by those patients who require high doses or are unable to use other methods of drug administration, such as metered-dose inhalers and spacer devices. This study has investigated only 3 nebulizers of the very many available to physicians. The detailed assessment took some months to complete and it would not have been practical for us to increase the number of nebulizers in the study. For this reason, we feel that the manufacturers, or a dedicated independent facility, should assess new inhalational drug delivery devices before they are marketed. We have demonstrated differences in nebulizer output with different nebulizers and different patient respiratory patterns. These factors should all be included in a standard nebulizer assessment. For instance, the output of budesonide from the Ventstream nebulizer was flow dependent, increasing by 50% at the adult breathing pattern. This may explain the relatively large budesonide delivery to the MSLI under constant flow conditions. Had the assessment not included breathing simulation, this study may have concluded that there is no difference in budesonide output between the Ventstream and the other nebulizers. Current standards for the assessment of nebulizers do not include the effect of breathing pattern on nebulizer function, and this needs to be addressed by regulatory authorities.

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