# The Influence of Breathing Pattern During Nebulization on the Delivery of Arformoterol Using a Breath Simulator

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**BACKGROUND: Patients with obstructive airway conditions, including chronic obstructive pulmonary disease (COPD), use nebulizers for drug delivery. Tidal breathing patterns employed by patients during nebulized drug delivery may vary. It is unclear whether different breathing patterns affect the emitted quantity of nebulized drug. This in vitro study evaluated whether different tidal breathing patterns that encompass a range that could be observed in COPD patients influence the emitted amount of nebulized arformoterol. METHODS: Breath-simulation experiments used a Pari LC Plus nebulizer in combination with the Duraneb 3000 portable aerosol system. Four breathing patterns that could represent a range of tidal volumes and inspiratory and expiratory times observed in patients with COPD were studied. The amount of arformoterol on the inspiratory and expiratory filters, and the residual amount in the nebulizer bowl were determined via high-pressure liquid chromatography. Results are expressed as a percent of the nominal dose (15 μg in 2 mL). RESULTS: The total amount of arformoterol on the inspiratory filter increased with a longer inspiratory phase of tidal breathing (ranging from 8.0% to 13.1%), while the expiratory filter dose remained similar (7.9% to 8.7%). The total emitted dose (expiratory and inspiratory amounts combined) for all patterns was 16.0% to 21.1% of the nominal dose. Retained arformoterol amount (not emitted) ranged from 55.9% to 62.3% of the nominal dose. CONCLUSIONS: These breath-simulation experiments suggest that only about 20% of the nominal 15-μg arformoterol dose was emitted from the nebulizer apparatus with each of the 4 tidal breathing patterns studied, and that a longer inspiratory phase was associated with greater inhaled dose.** *Key words: arformoterol, nebulizer, aerosol, breath-simulation, emitted dose*. [Respir Care 2009;54(11):1488–1492. © 2009 Daedalus Enterprises]

## Introduction

Delivery of inhaled airway medications via nebulization may be of benefit to patients with chronic obstructive pulmonary disease (COPD), in particular those who cannot coordinate deep inhalation required for effective actuation of a single-breath inhaler, those who cannot manipulate hand-held devices, and those who cannot achieve a minimum inspiratory effort, velocity or duration for adequate drug delivery via dry-powder inhaler.[1] Nebulizer design, flow rate requirement, solution properties, and respirable fraction are all variables that could influence inhaled drug delivery and effectiveness. In addition, a subject's breathing pattern during nebulization may impact the quantity of drug inhaled.[2] In vivo testing of nebulizer performance may be affected by the variability in tidal volumes and flow rates, both within and between subjects.[3] Prior studies have reported that in vivo aerosol delivery to the mouth from a jet nebulizer can be estimated using in vitro simulation of actual tidal breathing patterns.[4-6] For aqueous solutions, previous laboratory studies of nebulizer differ-

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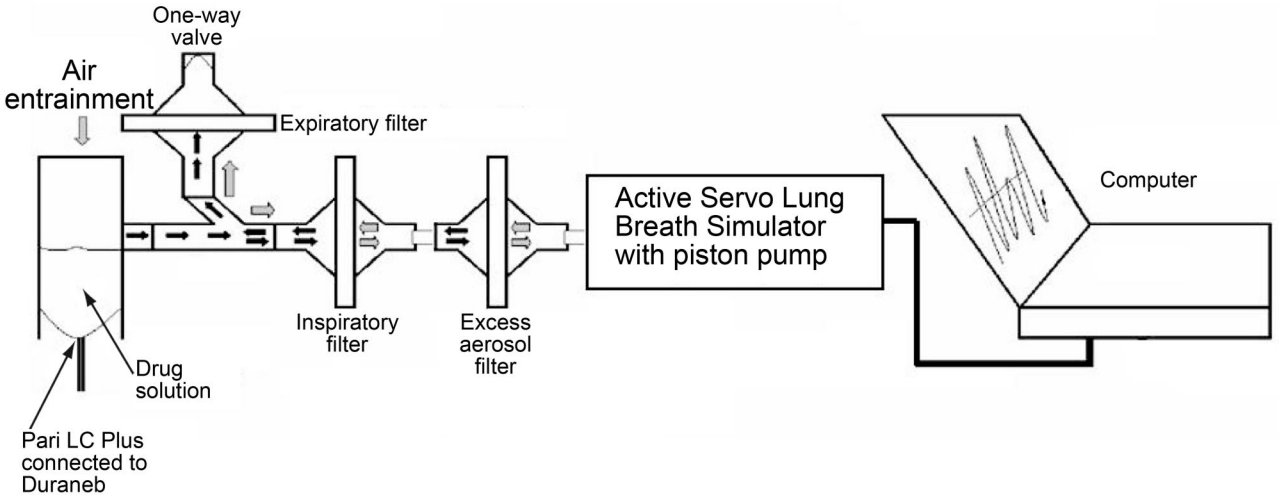


Fig. 1. Experimental setup for breath simulation.

ences in quantity of emitted drug have been correlated with differences in aerosol inhaled and lung deposition in patients.[7,8]

Arformoterol inhalation solution is a long-acting bronchodilator of the $\beta_2$-agonist class and is approved for the twice-daily treatment of bronchoconstriction in patients with COPD. The Pari LC Plus nebulizer, used in phase III arformoterol trials,[9,10] is a breath-enhanced nebulizer that contains a set of inspiratory and expiratory valves. These valves allow ambient air to be entrained into the nebulizing chamber during the inspiratory phase when the patient's flow exceeds the driving flow of the device.[11] During the expiratory phase, exhaled breath is directed out through a one-way valve.

This study investigated the effect of in vitro breathing patterns, generated by a breath simulator, on the inspiratory filter, the expiratory filter, and total amount of arformoterol solution emitted from a nebulizer. We sought to determine whether the emitted amount of arformoterol inhalation solution by nebulization was impacted by different tidal breathing patterns that could be seen in subjects with COPD.

## Methods

### Nebulizer and Drug

A portable aerosol system consisting of a reusable breath-enhanced nebulizer (Pari LC Plus, Pari Respiratory Equipment, Midlothian, Virginia) and a compressor (Duraneb 3000, Pari Respiratory Equipment, Midlothian, Virginia) at a mean flow rate of 3.3 L/min were used. For all measurements, the nebulizer was loaded with 2 mL (15 $\mu$g) of arformoterol nebulizing solution (Brovana, Sepracor, Marl borough, Massachusetts).

### Drug Assay

Chemical analysis of arformoterol was performed via high-pressure liquid chromatography. The technique used 16:84 (v/v) Acetonitrile, 50 mM $KH_2PO_4$, pH 3.85, as mobile phase, and a YMC Pack Pro C18 column (Waters, Milford, Massachusetts). The flow rate was set at 1.5 mL/min. Electrochemical detection (Coulochem II, ESA, Chelmsford, Massachusetts) was used for quantifying arformoterol. The limit of quantitation of the electrochemical assay was 5 nanograms per gram.

### Breath-Simulation Apparatus and Procedure

Breathing patterns were simulated using a computerized breathing simulator (Active Servo Lung ASL 5000, Ing-Mar Medical, Pittsburgh, Pennsylvania) (Fig. 1). A series of breath-simulation tidal patterns were selected to cover a range that could be seen in COPD patients, and included varied tidal volumes, breath frequencies, peak inspiratory flow rates, and inspiratory-to-expiratory time ratios (Table 1). The mouthpiece of the Pari LC Plus was removed and replaced by a Pari filter valve, set with filter pads (both Pari Respiratory Equipment, Midlothian, Virginia), which allowed collection of inspiratory and expiratory emitted arformoterol amounts. Between the Pari filter valve set and the Active Servo Lung, an additional Pari filter holder with filter was inserted to protect the breathing simulator from contamination. The nebulization time in these experiments was 8 min, which was similar to nebulization time in the clinical studies. The 8-min observation time allowed for collection and evaluation of emitted drug amount that included and went beyond the sputter point.

High-pressure liquid chromatography mobile-phase solution was used to extract arformoterol from both inspira-

Table 1. Breathing Patterns Used For Breath-Simulation Collections

| Breathing Parameter | Pattern 1 | Pattern 2 | Pattern 3 | Pattern 4 |
|---|---|---|---|---|
| Peak inspiratory flow (L/s) | 1.00 | 0.66 | 0.33 | 0.25 |
| Tidal volume (mL) | 650 | 500 | 350 | 300 |
| Frequency (breaths/min) | 10 | 12 | 12 | 15 |
| Total time per breath (s) | 6 | 5 | 5 | 4 |
| Inspiratory phase duration (s) | 1.3 | 1.5 | 2.1 | 2.4 |
| Expiratory phase duration (s) | 4.7 | 3.5 | 2.9 | 1.6 |
| Inspiratory-expiratory ratio | 1:3.6 | 1:2.3 | 1:1.4 | 1:0.7 |

Table 2. Results of Breathing Patterns Used For Breath-Simulation Collections

| Breathing Parameter | Pattern 1 | Pattern 2 | Pattern 3 | Pattern 4 |
|---|---|---|---|---|
| Inhaled dose (mean ± SD μg) | 1.2 ± 0.1 | 1.6 ± 0.3 | 1.6 ± 0.3 | 2.0 ± 0.5 |
| Inhaled dose (% of nominal dose) | 8.0 | 10.4 | 10.7 | 13.1 |
| Exhaled dose (mean ± SD μg) | 1.2 ± 0.4 | 1.3 ± 0.2 | 1.2 ± 0.3 | 1.2 ± 0.1 |
| Exhaled dose (% of nominal dose) | 8.0 | 8.7 | 7.9 | 7.9 |
| Ratio of inhaled dose to exhaled dose (mean ± SD) | 1.1 ± 0.4 | 1.2 ± 0.4 | 1.5 ± 0.5 | 1.6 ± 0.3 |
| Total mean delivered dose (μg, % of nominal dose)* | 2.4 (16.0) | 2.9 (19.1) | 2.8 (18.5) | 3.2 (21.1) |
| Total dose retained in nebulizer (mean ± SD μg) | 9.3 ± 0.4 | 9.2 ± 0.1 | 8.6 ± 0.5 | 8.4 ± 0.1 |
| Total dose retained in nebulizer (% of nominal dose) | 62.3 | 61.3 | 57.0 | 55.9 |
| Concentration of drug in nebulizer (mean ± SD μg/mL) | | | | |
| Start of experiment | 7.1 ± 0.1 | 7.1 ± 0.1 | 7.2 ± 0.0 | 7.2 ± 0.1 |
| End of experiment | 8.8 ± 0.2 | 8.5 ± 0.1 | 8.3 ± 0.2 | 8.1 ± 0.2 |
| Increase in concentration in nebulizer bowl from start to end of nebulization (mean ± SD %) | 23.4 ± 1.5 | 20.0 ± 1.7 | 15.2 ± 3.4 | 12.6 ± 3.1 |
| Retained solution volume (mean ± SD g) | 1.1 ± 0.03 | 1.1 ± 0.02 | 1.0 ± 0.05 | 1.0 ± 0.01 |
| Retained solution volume (% of nominal 2 mL) | 53.5 | 54.0 | 51.5 | 51.5 |
| Mass balance (%, range) | 86 (83–90) | 86 (83–90) | 82 (79–83) | 83 (80–86) |
| Time to sputter (min, range) | 2:24 (2:01–2:39) | 2:45 (2:17–3:02) | 2:58 (2:29–3:16) | 2:43 (2:23–3:05) |

* Total dose is the total amount of aerosolized medication leaving the nebulizer.

tory and expiratory filters and filter holders. In addition, the residual amount of arformoterol contained in the nebulizer bowl was determined via high-pressure liquid chromatography. The retained volume in the nebulizer bowl was measured by weight. The dose and volume values are reported, as well as the percentages of the nominal starting dose (15 μg arformoterol in 2 mL volume). For each breathing pattern, the experiment was performed 5 times, using the same nebulizer unit and compressor unit. Results presented are the means of the 5 replicates for each pattern.

## Results

The results of the breathing simulator experiments are summarized in Table 2 and Figure 2. The mean inhaled arformoterol amount as a percent of nominal dose increased with duration of the inspiratory phase of tidal breathing (8.0%, 10.4%, 10.7%, and 13.1% for breathing patterns 1–4, respectively). The difference in inhaled dose was significant for patterns 1 and 4 ($P = .002$). For exhaled dose there was no significant difference among breathing patterns ($P = .89$). For total delivered dose (in-

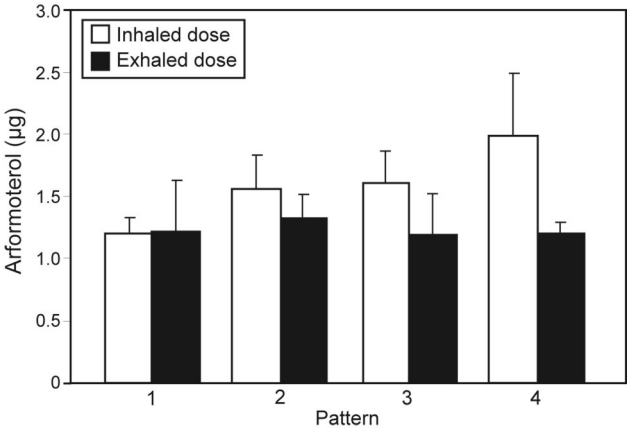


Fig. 2. Inspiratory and expiratory dose results from 4 breathing patterns (with standard deviations) (*P* value for inhaled dose for all 4 patterns .02, *P* value for exhaled dose for all 4 patterns .89).

spiratory filter plus expiratory filter) the difference between patterns 1 and 4 approached significance, although, overall there was no significant difference among the 4 breathing patterns ($P = .12$). There was a strong correlation between the decrease in the retained dose of arformoterol in the nebulizer as a percent of the nominal dose and increasing inspiratory duration (62.3%, 61.3%, 57.0%, and 55.9% for breathing patterns 1–4, respectively, $r^2 = 0.65$). The concentration of the solution in the nebulizer bowl at the end of the experiment increased by 23.4% for pattern 1, 20.0% for pattern 2, 15.2% for pattern 3, and 12.6% for pattern 4, from that at the start of the experiment (see Table 2). There was a good correlation between higher inhaled dose and reduction in concentration increase of arformoterol remaining in the bowl ($r^2 = 0.46$). There was also a good correlation between increased inhaled dose and increased inspiratory phase duration ($r^2 = 0.38$). Mean sputter point was similar for all 4 breathing patterns, and occurred between 2:24 and 2:58 min.

## Discussion

The amount of drug emitted by a nebulizer can impact the clinical efficacy and safety. Aerosol output from a nebulizer is affected by multiple factors, including nebulizer design, drug formulation, the patient's tidal volume, and breathing pattern.[3,7] Usually a single breathing pattern is used for analysis of dose emitted by a nebulizer for regulatory purposes in drug development. However, this does not allow for identification of possible variability in drug delivery due to respiratory patterns specific to a certain disease condition or for individual patient variability.[6,12-20] To our knowledge this is the first reported study that examined the impact of breathing patterns on the emitted drug amount of a nebulized long-acting $\beta_2$-agonist used in treating patients with COPD.

These breath-simulation experiments with the Pari LC Plus nebulizer suggest that only a small proportion of the nominal 15-$\mu$g arformoterol dose is emitted. The total amount of arformoterol recovered from the inspiratory filter ranged from 1.2 $\mu$g to 2.0 $\mu$g, which represents 8.0% to 13.1% of the nominal dose. However, the 60% increase in the amount of arformoterol on the inspiratory filter, from 1.2 $\mu$g to 2.0 $\mu$g, between breathing patterns 1 and 4 was significant. The relatively small difference in inhaled dose of between 8.0% and 13.1% of the nominal dose indicates that, overall, the different breathing patterns did not substantially impact the quantity of arformoterol emitted during an 8-min period of nebulization. The inhaled dose reported here has been demonstrated in prior clinical trials to be an effective long-acting bronchodilator in the treatment of COPD.[9,10] This range of inhaled dose is less than previous reports that used healthy adult breathing patterns, and found that the Pari LC Plus emitted about 19–22% of the label dose.[19,21] Some of the differences between the studies may reflect the different breathing patterns used, but may also reflect the difference in experimental design. Although both prior studies used the same (2 mL)[21] or similar (2.5 mL)[19] nominal volumes, they used different methods for driving the aerosol delivery, which may have impacted the amount of inhaled dose. The total delivered dose (inspiratory plus expiratory filters) was between 16.0% and 21.1% of the nominal dose. In these experiments a minimum of 8.4 $\mu$g of arformoterol, or 56% of the nominal 15-$\mu$g dose, was retained in the bowl after nebulization with all breathing patterns studied.

Longer inspiration times correlated well with increases in inhaled dose, and strongly correlated with a reduction in the amount of arformoterol retained in the nebulizer. Consistent with this, there was also a good correlation between the inhaled dose and the amount of drug retained in the bowl. Prior studies similarly report that higher inspiration times[5] and lower breathing rates[12] increased the amount of inhaled dose from breath-enhanced jet nebulizers. Whether the observed differences in these in vitro experiments are predictive of differences in the amount of drug emitted to the mucosal or airway surfaces is not known. Prior work has indicated a correlation between in vivo and in vitro breathing patterns.[4,5] These results suggest that longer inspiratory times during nebulized drug administration in patients with COPD may lead to increases in the emitted and delivered amount of drug.

Several conditions of this study should be considered in the interpretation of these results. The reported delivery differences related to breathing patterns may be specific to the Pari LC Plus nebulizer/Duraneb 3000 system, and may not be predictive of drug emitted by other nebulizer/compressor systems, as the overall performances of nebulizer systems are known to range widely.[2] Estimates of the amount of a drug emitted to a patient in vivo are most accurate when the experimental conditions are as clinically relevant as possible.[7] In these experiments, 4 different tidal breathing patterns, representing a range of patterns that could be observed in patients with COPD, were evaluated. However, for each pattern, each breath was identical to the next. Results could be different for breathing patterns outside the range of those tested, or for patterns that vary from breath to breath over a given nebulization time, as might occur with patients' tidal breathing. In some in vitro studies, nebulizers were evaluated by time of sputter,[22,23] at which time the quantity of aerosol generated diminishes. Sputter occurred after 2–3 min in this study, which is shorter than that seen in clinical trials.[9,10] This is explained in part by the experimental design, which kept the nebulizer bowl stationary during the entire period of nebulization. In clinical use, patient movement results in reintroduction of condensate into the bowl and longer

time to sputter. Finally, these results estimate the amount of aerosolized drug output by the nebulizer apparatus, and not that deposited in the lung or airways. Therefore, conclusions made from these experiments with respect to actual drug delivery to patients must be made with caution, as delivery of the biological dose to the airways and lungs is more complex and dependent on additional factors, such as aerosol particle size, airway geometry, and regional airway ventilation.

## Conclusions

In summary, this in vitro breath-simulation study showed that there was a good correlation between longer inspiratory times and increased amount of arformoterol inhalation solution available for inhalation into the lung. There was a strong correlation between longer inspiration times and a reduction in the amount of drug retained in the nebulizer bowl. The total inhaled arformoterol dose was approximately 8.0% to 13.1% of the nominal dose, and this amount was not substantially impacted by different in vitro tidal breathing patterns. It is reasonable to speculate that these observations may also apply to other nebulized solutions.

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