Aerosol Delivery of Recombinant Human DNase I: In Vitro Comparison of a Vibrating-Mesh Nebulizer With a Jet Nebulizer

Jeremy C Johnson DO, J Clifford Waldrep PhD, Jennifer Guo MD, and Rajiv Dhand MD FAARC

BACKGROUND: Inhaled recombinant human DNase I (rhDNase) improves clearance of visco-elastic secretions in patients with cystic fibrosis. Because of their portability, newer-generation vibrating-mesh nebulizers offer greater convenience for the patient, but their efficiency in delivering rhDNase has not been determined. METHODS: We compared a newer-generation vibrating-mesh nebulizer (Omron MicroAir) to a Pari LC+ with the Pari ProNeb Ultra compressor (a commonly employed rhDNase administration system). With the Next Generation Pharmaceutical Impactor, we determined aerosol particle distribution. We also measured mass output efficiency, nebulization time, and mass of rhDNase that deposited on a filter during simulated breathing. RESULTS: The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of aerosol from the MicroAir (MMAD 4.3 μm, GSD 2.8 μm) was equivalent to that from the Pari LC+ (MMAD 4.2 μm, GSD 2.7 μm). During simulated breathing the MicroAir had a higher total mass output efficiency (88%) than the Pari LC+ (68%) ($P < .001$), and total nebulization time was shorter with the MicroAir (6.1 min vs 7.2 min, $P = .03$). When nebulized to dryness, the mass of rhDNase delivered to the filter was comparable with the MicroAir (1.30 ± 0.4 mg) and Pari LC+ (1.21 ± 0.05 mg). CONCLUSION: The MicroAir could be employed as a portable nebulizer for rhDNase therapy in patients with cystic fibrosis. *Key words: nebulizer, vibrating mesh, aerosol, inhalation, cystic fibrosis, recombinant human DNase I.* [Respir Care 2008;53(12):1703–1708. © 2008 Daedalus Enterprises]

Introduction

Respiratory complications, including persistent bacterial infections, recurrent exacerbations, and lung destruction,[1,2] are a major cause of morbidity and mortality in patients with cystic fibrosis (CF). Deoxyribonucleic acid (DNA) released locally by degenerating neutrophils, which accumulate in response to infection in the airways, is highly viscous and contributes to development of chronic airway obstruction.[3,4] Inhaled recombinant human DNase I (also known as rhDNase, dornase alfa, and Pulmozyme) hydrolyzes extra-cellular DNA in purulent secretions of patients with CF, reduces the secretions' visco-elasticity, and improves secretion clearance.[5] A few investigators have found that rhDNase nebulized via a conventional jet nebulizer is safe and improves symptoms and pulmonary function in patients with CF.[6-11]

With advances in device technology, a plethora of aerosol devices and drug formulations are available for clinical use.[12-17] For example, recent advances in nebulizer design have introduced small portable devices based on either a vibrating mesh or a plate with multiple apertures.[12,16,17] The vibrating-mesh nebulizers are being tested for various clinical indications.[18,19] To the best of our knowledge, these newer nebulizers have not been evaluated for aerosol delivery of rhDNase.

Jeremy C Johnson DO, J Clifford Waldrep PhD, Jennifer Guo MD, and Rajiv Dhand MD FAARC are affiliated with the Division of Pulmonary, Critical Care, and Environmental Medicine, University of Missouri-Columbia, and Harry S Truman Memorial Veterans Affairs Hospital, Columbia, Missouri.

This research was partly supported by Omron Healthcare (which provided the MicroAir nebulizers), the United States Veterans Affairs Research Service, the Department of Internal Medicine, University of Missouri, Columbia, and the Missouri Research Foundation. The device and drug manufacturers were not involved in designing the study, collecting, analyzing, or interpreting the data, or preparing the manuscript. The authors report no other conflicts of interest related to the content of this paper.

Correspondence: Rajiv Dhand MD FAARC, Division of Pulmonary, Critical Care, and Environmental Medicine, University of Missouri-Columbia, MA-421 Health Sciences Center, DC043.00, 1 Hospital Drive, Columbia MO 65212. E mail: dhandr@health.missouri.edu.

We compared the delivery of rhDNase from the MicroAir nebulizer (Omron Healthcare, Bannockburn, Illinois) to that from the Pari LC+ disposable jet nebulizer (Pari Respiratory Equipment, Midlothian, Virginia) with the Pari ProNeb Ultra compressor (Pari Respiratory Equipment, Midlothian, Virginia). We choose this jet nebulizer/compressor system because it is one of the systems recommended by the manufacturer of rhDNase for delivery of rhDNase[20] and is commonly employed in clinical practice for this application.

Methods

Devices and Drug

The MicroAir nebulizers (lots 32A and 44A) were gifts from Omron Healthcare. We purchased the Pari LC+ nebulizer and Pari ProNeb Ultra Compressor. In all the tests we used a commercial preparation of rhDNase (Pulmozyme, Genentech, South San Francisco, California) that comes in 2.5-mL ampules (1.0 mg/mL, 2,500 U) of a solution for inhalation formulated in 8.77 mg/mL sodium chloride, or 0.15 mg/mL calcium chloride dehydrate and sterile water for injection.

Deoxyribonuclease Activity of rhDNase

Enzymatic activity in nebulized rhDNase samples was determined by the Kunitz method.[21,22] The reaction rate (maximum enzyme velocity) was measured by the rate of hydrolysis of DNA, measured spectrophotometrically at 260 nm and 25°C. The stock DNA substrate solution contained 4 mg of salmon testes DNA in 0.5 M acetate buffer, pH 5.0, and 0.05 M magnesium chloride solution. Aliquots of the test samples collected from the cascade impactor (Next Generation Pharmaceutical Impactor [NGI], MSP Corporation, Shoreview, Minnesota) plates or inhalation filters were added to 1 mL DNA test solution, and the reaction rate was measured over 6 min. The calculated maximum enzyme velocity reaction rate was used to determine DNase activity. The sensitivity of the assay was 10 units, based on a 3-point standard curve prepared with 1,000 units/mL rhDNase, as reported for each manufactured test lot, with an assay variability of $< 10\%$.

Aerosol Particle Size Distribution

Aerodynamic particle sizing of the rhDNase aerosols was via cascade impaction with the NGI, as previously described.[18,23] rhDNase I aerosols were generated from the MicroAir nebulizer and the Pari LC+ nebulizer with one ampule of rhDNase and drawn through the cascade impactor with a vacuum pump at 15 L/min, at a controlled temperature of 23–24°C, and at 50 ± 5% relative humidity. The nebulizer was connected to the induction port of the NGI with a mouthpiece adapter. The positions of the devices simulated those used in clinical practice, and the aerosols from both devices were entrained into the NGI in line with the horizontal axis of the induction port. Aerosols were sampled for 2 min, and the experiments were repeated in triplicate or quadruplicate with each device. The impacted rhDNA was eluted from the NGI plates after agitation for 5 min with 10 mL of ultrapure water.

After determination (via Kunitz assay) of the enzyme concentrations deposited in the induction port, on each NGI stage, and on the final filter, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the aerosol were calculated on a log probability scale on which the effective cutoff diameter is on the ordinate and the cumulative percent less than the size range (by concentration) is on the abscissa (KaleidaGraph 3.51, Synergy Software, Reading, Pennsylvania). The MMAD and GSD were determined by the rhDNase concentration distributed within the array of aerosol droplets. The respirable fraction was calculated as the percentage of aerosol particles < 5.0 μm, and the fine-particle fraction was calculated as the percentage of aerosol drug particles < 3.3 μm.[24,25]

Measurement of Mass Delivered to the Filter

To determine the amount of nebulized rhDNase delivered to the filter, samples were collected in a simulated human lung system.[26] We used a respirator pump (Harvard Apparatus, Dover, Massachusetts) and employed an exhalation valve, such that aerosol samples were collected onto a bacteria/virus filter (Respirgard, Marquest Medical Products, Englewood, Colorado) (Fig. 1). The exhaled air was vented into a hood.

Total rhDNase delivered from the MicroAir and Pari LC+ with 2.5 mL rhDNase was collected on the filters (5 of each), at 12 breaths/min with tidal volume of 500 mL, and inspiratory-expiratory ratio of 1:1. The MicroAir was held in a horizontal position (see Fig. 1). Both devices were connected to the proximal port of the filter. The MicroAir was connected to the filter with a mouthpiece adapter and mouthpiece. The Pari LC+ was connected to the filter with a mouthpiece adapter. The nebulization began immediately before the inspiratory stroke of the pump piston. The nebulizers were run to dryness. Because the solution remaining in the jet nebulizer gave inconsistent results with the enzyme assay, we determined mass output of rhDNase based on nebulizer weights before and after nebulization. The nebulizer efficiency was calculated by the following formula:

$$[(\text{pre-nebulization weight} - \text{post-nebulization weight})/\text{pre-nebulization weight}] \times 100$$

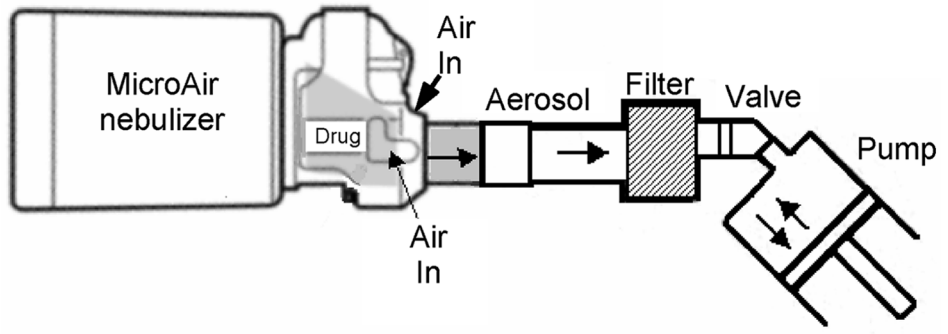

Fig. 1. Test setup.

Table 1. Aerosol Characteristics of the Nebulizers

	MMAD (μm)*	Geometric Standard Deviation†	Respirable Fraction‡ %	Fine Particle Fraction§ %
MicroAir (mean ± SD)	4.3 ± 0.5	2.8 ± 0.3	56.7 ± 3.0	40.7 ± 6.1
Pari LC+ (mean ± SD)	4.2 ± 0.5	2.7 ± 0.4	56.3 ± 4.1	44.0 ± 6.2

* $P = .77$
† $P = .69$
‡ $P = .92$
§ $P = .66$
MMAD = mass median aerodynamic diameter

The rhDNase deposited on the filter was extracted via agitation for 5 min with 10 mL ultrapure water, and the DNase activity was determined via Kunitz assay.[21,22]

Data Analysis

We used statistics software (Instat 3, GraphPad Software, La Jolla, California) to tabulate the data and calculate the mean ± SD values. We analyzed the MMAD, GSD, mass delivered to the filter, respirable fraction, and fine-particle fraction data with 2-tailed paired *t* tests. *P* values $< .05$ were considered significant.

Results

The experiments were designed to measure aerosol delivery of rhDNase from the MicroAir and the Pari LC+ with Pari ProNeb Ultra compressor.

Aerosol Particle Size and Distribution

Table 1 compares the aerosol characteristics of rhDNase nebulized with the MicroAir versus with the Pari LC+, via NGI analysis. Both devices produced a heterodisperse aerosol with comparable distribution of particle sizes (MMAD 4.3 ± 0.5 μm and GSD 2.8 ± 0.3 μm with MicroAir vs MMAD 4.2 ± 0.5 μm and GSD 2.7 ± 0.4 μm with Pari LC+). The 2 devices' aerosol particle distributions in the respirable range were not statistically different. The respirable fraction from the MicroAir was 56.7 ± 3.0%, compared to 56.3 ± 4.1% from the Pari LC+. The fine-particle fraction from the MicroAir was 40.7 ± 6.1%, compared to 44.0 ± 6.2% from the Pari LC+ (see Table 1).

Nebulization Time, Output Efficiency, and Mass Delivered to the Filter

Table 2 shows the devices' nebulization characteristics with a simulated breathing system. With a charge dose of 2.5 mL of rhDNase, the MicroAir nebulized to dryness in 6.1 min, versus 7.2 min with the Pari LC+ ($P = .03$). As determined via nebulizer weight before and after nebulization, the total drug output from the MicroAir (2.2 ± 0.03 mg) was higher than that from the Pari LC+ (1.7 ± 0.1 mg, $P < .001$) (see Table 2). This resulted in higher nebulizer efficiency with the MicroAir (88%) than with the Pari LC+ (68%, $P < .001$), mainly due to the MicroAir's lower residual volume. There was, however, no significant difference in the amount of rhDNase deposited on the filter (MicroAir 1.30 mg, Pari LC+ 1.21 mg) (see Table 2).

Discussion

The present study compared the MicroAir to a currently utilized breath-enhanced nebulizer (Pari LC+) for deliv-

Table 2. Nebulizer Characteristics With a Simulated Breathing System

	Time to Dryness (min)*	Output Efficiency (%)†‡	Total Nebulizer Output (mg)†‡	Filter Mass (mg)§‖
MicroAir (mean ± SD)	6.1 ± 0.5	88.0 ± 1.7	2.2 ± 0.03	1.30 ± 0.4
Pari LC+ (mean ± SD)	7.2 ± 0.5	67.8 ± 4.6	1.7 ± 0.1	1.21 ± 0.05

* $P = .03$
† Output is determined via nebulizer weight difference before and after nebulization.
‡ $P < .001$
§ Filter mass = amount of rhDNase deposited on filter, assayed via enzyme activity.
‖ $P = .24$

ery of rhDNase. We found that the MicroAir is a convenient and efficient hand-held aerosol generator that provides comparable rhDNase output to Pari LC+.

The MicroAir has several distinctive features. It is a battery-operated, portable device that employs a vibrating mesh for aerosol generation, and it produces a fine aerosol with a low velocity.[18,27] The MicroAir rapidly aerosolizes drug solutions, so 2.5 mL of rhDNase is nebulized in a shorter time than with Pari LC+ with the ProNeb Ultra compressor. The aerosol particle distribution analysis with the NGI revealed that both devices produced heterodisperse aerosols with similar particle sizes. Both MicroAir and Pari LC+ generate aerosol particles within the range required for relatively uniform delivery to the airways within the lung.[24-26,28]

In the present study we employed an enzymatic method to assay the rhDNase and determine the MMADs and GSDs. Previous studies[29-31] with jet nebulizers employed different cascade impaction techniques[30] or laser diffraction,[29,31] and no previous studies have been performed with rhDNase delivered by a vibrating-mesh nebulizer. Recently, the European Standard (EN 13544-1) was developed to standardize laboratory methods for determination of particle size in nebulized aerosols.[32] In our study, which was conducted according to the guidelines of the European Nebulizer standard,[32] the distribution of rhDNase particles in the aerosol generated by both devices was equivalent.

In related studies, previous investigators employed jet nebulizers and various techniques to size rhDNase aerosols.[29-31] Cipolla and colleagues[30] used dry weights to determine rhDNase deposition on the impactor plates. Other investigators employed laser diffraction to size aerosol particles.[29,31] Those studies assumed that rhDNase and excipients distribute equally among all aerosol particles, but the amount of rhDNase in various fractions cannot be directly determined by the techniques employed in previous studies.[29-31] In contrast, we determined rhDNase deposition by direct measurement of its activity in various particle fractions. In our previous study with the MicroAir we found that various laboratory techniques yielded different results for particle size distribution.[18] Other factors, such as the ionic strength, density, surface tension, and viscosity of the solution, also influence drug output and particle size from a nebulizer that employs a vibrating aperture plate to generate aerosol.[33] Despite the differences in test methods, our results (MMAD approximately 4.2 μm) are comparable to values obtained by previous investigators. Cipolla and colleagues reported an MMAD range of 2.6–5.6 μm for aerosols of rhDNase generated by various Pari nebulizers.[30] With other jet nebulizers the particle size distribution of rhDNase aerosols have also been comparable to our results.[29,31]

In our study the Pari LC+ aerosolized only 68% of the rhDNase, whereas 88% of the drug placed in the MicroAir was aerosolized. However, despite the difference in mass output, the mass of rhDNase delivered to the filter in the simulated breathing experiment was comparable with the MicroAir and Pari LC+, which suggests a higher rhDNase deposition in the inspiratory tubing en route to the filter with the MicroAir. The similarity in the mass of rhDNase on the filter and the particle size of the aerosols generated by the 2 devices suggests that a comparable amount of rhDNase would be available to the patient with either device, and the difference in pulmonary deposition of rhDNase between the 2 devices is probably not clinically important. Ideally, these observations would be confirmed with scintigraphic studies with radiolabeled rhDNase in patients with CF. A study with healthy adults and the Pari LC+ found very close agreement between the respirable fraction of tobramycin measured via laser diffraction and the in vivo respirable fraction measured via scintigraphy.[34] However, in general, in vitro assessments tend to overestimate lung deposition.[24,28] In previous investigations, clinical response to inhaled rhDNase was similar with jet nebulizers with comparable performance and aerosol characteristics.[31,35] The similarity in aerosol characteristics and mass delivered to the filter with MicroAir and Pari LC+ suggests that clinical response would be comparable with these 2 devices.

The Kunitz assay gave unreliable results when assaying for rhDNase in the solution remaining in the jet nebulizer after nebulization, so we had to resort to weighing the nebulizers to determine total drug output. Because of their

ampipathic nature, protein molecules tend to occupy the surface in protein solutions, and they may undergo surface denaturation during jet nebulization.[36] In contrast to our findings, earlier investigators[29] found that enzymatic activity of rhDNase was preserved in the solution remaining in the nebulizer cup after jet or ultrasonic nebulization. However, the assay employed by Cipolla and co-workers[29] did not directly assess the action of rhDNase on DNA.

CF is a chronic illness that requires routine and frequent treatments. Similar to other jet nebulizers,[29,36] rhDNase delivered to the filter retains its activity after nebulization with MicroAir. In patients with CF, MicroAir would be expected to produce clinical improvement comparable to that with other jet nebulizers.[6-11] Because of its higher drug-output rate, the MicroAir takes less time to nebulize rhDNase than does the Pari LC+ (see Table 2). MicroAir's faster nebulization rate, compactness, and portability might improve patient adherence to treatment. MicroAir is also quieter than a conventional jet nebulizer.

Limitations

Our testing was under controlled laboratory conditions and therefore did not consider potential problems with patient coordination and irregular breathing patterns that may be encountered in clinical use.

We did not test the MicroAir over an extended period, as would be required in clinical practice.

The cost of the MicroAir (approximately $200) is higher than that of Pari LC+ and compressor (approximately $70). The MicroAir also needs rigorous cleaning; the manufacturer recommends disassembly, immersion in water, and disinfection with 10% distilled vinegar after each use to prevent clogging of the mesh apertures with drug particles, salts, or microbes. Cleaning and reassembling the MicroAir requires several minutes after each treatment. Aperture clogging was not a problem in our experiments, because we adhered to a cleaning regimen. The latest recommendation on cleaning the MicroAir (effervescent denture cleanser) might further reduce aperture clogging.

Conclusions

The MicroAir produces a fine-particle, heterodisperse rhDNase aerosol that is comparable to that from the Pari LC+. These 2 devices deliver comparable amounts of active rhDNase to the airways. The MicroAir has the advantages of higher efficiency, better portability, shorter drug-delivery time, and greater convenience of use, compared to conventional jet nebulizers. The MicroAir could be employed as a portable device for rhDNase therapy in patients with CF. Further studies are needed to determine MicroAir's clinical efficiency.

REFERENCES

1. FitzSimmons SC. The changing epidemiology of cystic fibrosis. J Pediatr 1993;122(1):1-9.
2. Konstan MW, Berge M. Current understanding of the inflammatory process in cystic fibrosis: onset and etiology. Pediatr Pulmonol 1997; 24(2):137-142.
3. Ratjen F, Döring G. Cystic fibrosis. Lancet 2003;361(9358):681-689.
4. Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. Am J Respir Crit Care Med 2003;168(8):918-951.
5. Shak S, Capon DJ, Hellmiss R, Marsters SA, Baker CL. Recombinant human DNase 1 reduces the viscosity of cystic fibrosis sputum. Proc Natl Acad Sci USA 1990;87(23):9188-9192.
6. Ramsey BW, Astley SJ, Aitken MA, Burke W, Colin AA, Dorkin HL, et al. Efficacy and safety of short-term administration of aerosolized recombinant human deoxyribonuclease in patients with cystic fibrosis. Am J Respir Crit Care Med 1993;148(1):145-151.
7. Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. N Engl J Med 1994;331(10):637-642.
8. Shah PL, Scott SF, Geddes DM, Hodson ME. Two years experience with recombinant human DNase I in the treatment of pulmonary disease in cystic fibrosis. Respir Med 1995;89(7):499-502.
9. McCoy K, Hamilton S, Johnson C; Pulmozyme Study Group. Effects of 12-week administration of dornase alfa in patients with advanced cystic fibrosis lung disease. Chest 1996;110(4):889-895.
10. Quan JM, Tiddens HA, Sy JP, McKenzie SG, Montgomery MD, Robinson PJ, et al; Pulmozyme Ealy Intervention Trial Study Group. A two-year randomized placebo-controlled trial of dornase alpha in young patients with cystic fibrosis with mild lung function abnormalities. J Pediatr 2001;139(6):813-820.
11. Hodson ME, Mckenzie S, Harms HK, Koch C, Mastella G, Navarro J, Strandvik B; Investigators of the Epidemiologic Registry of Cystic Fibrosis. Dornase alpha in the treatment of cystic fibrosis in Europe: a report from the Epidemiologic Registry of Cystic Fibrosis. Pediatr Pulmonol 2003;36(5):427-432.
12. Smaldone GC. Advances in aerosols: adult respiratory disease. J Aerosol Med 2006;19(1):36-46.
13. Hess D, Fisher D, Williams P, Pooler S, Kacmarek RM. Medication nebulizer performance. Effects of diluent volume, nebulizer flow, and nebulizer brand. Chest 1996;110(2):498-505.
14. Smaldone GC, LeSouef PN. Nebulization: the device and clinical considerations. In: Bisgaard H, O'Callaghan CO, Smaldone GC, editors. Drug delivery to the lung. New York: Marcel Dekker; 2002: 269-302.
15. Rau JL. The inhalation of drugs: advantages and problems. Respir Care 2005;50(3):367-382.
16. Smaldone GC. Assessing new technologies: patient-device interactions and deposition. Respir Care 2005;50(9):1151-1160.
17. Dhand R. Nebulizers that use a vibrating mesh or plate with multiple apertures to generate aerosol. Respir Care 2002;47(12): 1406-1418.
18. Waldrep JC, Berlinski A, Dhand R. Comparative analysis of methods to measure aerosols generated by a vibrating mesh nebulizer. J Aerosol Med 2007;20(3):310-319.
19. Dhand R, Sohal H. Pulmonary drug delivery system for inhalation therapy in mechanically ventilated patients. Expert Rev Med Devices 2008;5(1):9-18.
20. Package insert. Full prescribing information for Pulmozyme dornase alfa. San Francisco: Genentech; 1994.

21. Kunitz M. Crystalline deoxyribonuclease; isolation and general properties; spectrophotometric method for the measurement of deoxyribonuclease activity. J Gen Physiol 1950;33(4):349-362.
22. Kunitz M. Crystalline deoxyribonuclease; digestion of thymus nucleic acid; the kinetics of the reaction. J Gen Physiol 1950;33(4): 363-377.
23. Marple VA, Olson BA, Santhanakrishnan K, Roberts DL, Mitchell JP, Hudson-Curtis BL. Next generation pharmaceutical impactor: a new impactor for pharmaceutical inhaler testing. Part III: extension of archival calibration to 15 L/min. J Aerosol Med 2004;17(4):335-343.
24. Newman SP. How well do in vitro particle size measurements predict drug delivery in vivo? J Aerosol Med 1998;11(Suppl 1):S97-S104.
25. Sangwan S, Condos R, Smaldone GC. Lung deposition and respirable mass during wet nebulization. J Aerosol Med 2003;16(4):379-386.
26. Smaldone GC, Fuhrer J, Steigbigel RT, McPeck M. Factors determining pulmonary deposition of aerosolized pentamidine in patients with human immunodeficiency virus infection. Am Rev Respir Dis 1991;143(4 Pt 1):727-737.
27. Waldrep JC, Dhand R. Advanced nebulizer designs employing vibrating mesh/aperture plate technologies for aerosol generation. Curr Drug Deliv 2008;5(2):114-119.
28. Newman SP, Chan HK. In vitro/in vivo comparisons in pulmonary drug delivery. J Aerosol Med 2008;21(1):77-84.
29. Cipolla D, Clark AR, Chan HK, Gonda I, Shire SJ. Assessment of aerosol delivery systems for recombinant human deoxyribonuclease. STP Pharma Sciences 1994;4:50-62.
30. Cipolla D, Gonda I, Shire SJ. Characterization of aerosols of human recombinant deoxyribonuclease I (rhDNase) generated by jet nebulizers. Pharma Res 1994;11(4):491-498.
31. Shah PL, Scott SF, Geddes DM, Conway S, Watson A, Nazir T, et al. An evaluation of two aerosol delivery systems for rhDNase. Eur Respir J 1997;10(6):1261-1266.
32. Boe J, Dennis JH, O'Driscoll BR, Bauer TT, Carone M, Dautzenberg B, et al; European Respiratory Society Task Force on the use of nebulizers. European Respiratory Society guidelines on the use of nebulizers. Eur Respir J 2001;18(1):228-242.
33. Zhang G, David A, Wiedemann TS. Performance of the vibrating membrane aerosol generating device: Aeroneb micropump nebulizer. J Aerosol Med 2007;20(4):408-416.
34. Coates AL, MacNeish CF, Dinh L, MacNeish CF, Rollin T, Gagnon S, et al. Accounting for radioactivity before and after nebulization of tobramycin to insure accuracy of quantification of lung deposition. J Aerosol Med 2000;13(3):169-178.
35. Fiel SB, Fuchs HJ, Johnson C, Gonda I, Clark AR. Comparison of three jet nebulizer aerosol delivery systems used to administer recombinant human DNase I to patients with cystic fibrosis. Chest 1995;108(1):153-56.
36. Hung JC, Hambleton G, Super M. Jet nebulizing systems for recombinant human DNase I. Arch Dis Child 1994;71(6):558-559.