Suitability of Caspofungin for Aerosol Delivery

Physicochemical Profiling and Nebulizer Choice

Annie Wong-Beringer, PharmD; Maria Polikandritou Lambros, PhD; Paul M. Beringer, PharmD; and David L. Johnson, PhD

Background: Aerosolized antifungal therapy is a promising route of drug delivery for pulmonary aspergillosis due to attainment of high localized concentrations. Caspofungin, a new antifungal agent with proven efficacy against invasive aspergillosis, has ideal potential for aerosolization.

Study Objective: To examine in vitro the suitability of caspofungin for aerosol administration by characterizing factors that influence efficacy and airway tolerance of aerosol delivery: physicochemical properties, aerodynamics of drug particles, and efficiency of nebulizing systems.

Design: Physicochemical characteristics of caspofungin solutions (10 mg/mL and 30 mg/mL) were analyzed: osmolality, pH, viscosity, and surface tension. A time-of-flight aerosol spectrometer API Aerosizer was used to determine aerosol particle size and distribution. Drug output was quantified by high-performance liquid chromatography assay. Nebulizer efficiency was measured by drug output and respirable fraction (percentage of aerosolized particles with an aerodynamic diameter < 5 μm) and compared among three jet nebulizer/compressor systems: device 1, Micromist (Hudson RCI; Temecula, CA)/Pulmo-Aide (model 5650D; DeVilbiss; Somerset, PA); device 2, Sidestream MS 2400/Envoy model IRC 1192 (Invacare; Elyria, OH); and device 3, Pari LC Star/Proneb Ultra (Pari Respiratory Equipment; Midlothian, VA).

Measurements and results: Caspofungin requires 0.9% NaCl rather than sterile water as the diluent and addition of 0.3N NaOH buffer to adjust acidity of solutions (pH 6.17 to 6.26) in order to achieve optimal physicochemical properties for airway tolerability (osmolality, 150 to 550 milliosmol per kilogram; chloride ion, 31 to 300 mmol/L; and pH 7.4). The drug output rate increased with higher concentrations of drug solution: device 1, 4.0 mg/min vs 12.5 mg/min; device 2, 5.4 mg/min vs 14.7 mg/min; and device 3, 2.3 mg/min vs 12 mg/min, respectively. The percentage of particles within the respirable range varies depending on device and concentration of drug solutions (10 mg/mL vs 30 mg/mL): device 1, 85% vs 38%; device 2, 44% vs 57%; and device 3, 83% vs 93%, respectively.

Conclusion: Caspofungin solution with adjustments appears to have physicochemical and aerodynamic characteristics suitable for aerosolization when used with either the Pari LC Star/Proneb Ultra or Micromist/Pulmo-Aide devices. Further in vivo testing is warranted.

Key words: aerosol; antifungal; Aspergillus; echinocandin; jet nebulizer; nebulization; time-of-flight aerosol spectrometer

Abbreviations: cst = centistoke; IPA = invasive pulmonary aspergillosis; MMAD = mass median aerodynamic diameter; mosm = milliosmol; psi = pounds per square inch; RF = respirable fraction

Invasive pulmonary aspergillosis (IPA) is a major infectious complication among transplant recipients and patients receiving cancer chemotherapy. Antifungal prophylaxis against IPA is the key to improving patient outcomes, since associated mortality is up to 85% once infection is established. Aerosolization of antifungal therapy is a promising route of drug delivery for prophylaxis due to attain-
ment of high drug concentration in the lungs, which are the primary portal of entry for fungi. In addition, topical administration affords patient convenience and minimizes systemic toxicities. Amphotericin B, the “gold standard” antifungal agent, has been administered as an aerosol for prophylaxis in immunocompromised hosts; however, results have been conflicting possibly due to heterogeneity in the study populations, physicochemical properties of aerosol formulations, and nebulizing systems used.2–7

Caspofungin is the first agent of a new class, the echinocandins, to have received US Food and Drug Administration approval for the treatment of aspergillosis in patients who are refractory to or intolerant of amphotericin B and/or itraconazole, as well as for invasive candidiasis and febrile neutropenia.8–11 An echinocandin derivative similar to caspofungin (L-693,989) was shown to be effective in delaying mortality from IPA when administered as an aerosol prophylactically to rats12; however, to our knowledge, aerosolized echinocandin has not been evaluated in humans. Caspofungin is a promising agent to consider for aerosol administration, in that the drug not only has significant activity against Aspergillus but also other fungal pathogens causing pneumonia in the immunocompromised hosts such as Pneumocystis carinii.8,9 In addition, it is relatively less toxic compared to other available agents. Its aqueous-only IV dosage form can potentially be administered as an aerosol to patients for prophylaxis in the ambulatory setting, particularly when the drug is inadequately absorbed following oral administration.8,9

The achievement of maximal efficacy for aerosol drug delivery has been shown to depend in part on the selection of a drug solution with optimal physical properties for aerosolization and an ideal nebulizing system.13–17 Physical properties such as the pH, osmolality, viscosity, and chloride ion concentration of solution may affect nebulization rates, lung deposition as related to particle size, and airway tolerability.13 For example, a hypotonic or hypertonic solution in large amounts can result in mucosal irritation, while cough is regularly elicited by inhalation of solutions with an osmolality < 100 or > 1,100 milliosmol (mosm)/kg.13,18 However, increasing viscosity as a result of increasing drug concentration may dramatically decrease the nebulization rate.13 In addition, the distribution of aerosol particle size generated determines the pattern of drug deposition in the respiratory tract.15,17 Aerosol particle size measured in mass median aerodynamic diameter (MMAD) of 1 to 5 µm is optimal because antibiotic delivery to the site of action (peripheral airways and the alveolar region of the lung) is maximized.15,17 Therefore, we determined the suitability of caspofungin solution for aerosol administration by evaluating the following: (1) selected physicochemical properties that may affect airway tolerability and nebulization efficiency, and (2) different commercial nebulizing systems on particle size distribution of the aerosols generated and output efficiency.

Materials and Methods

Caspofungin solutions were prepared from a commercially available formulation for physicochemical and stability measurements and nebulization experiments. Drug powder was reconstituted with 0.9% NaCl to make 10 mg/mL and 30 mg/mL solutions. Three commercial jet nebulizer systems were compared to determine the optimal system for delivery of caspofungin aerosols. Nebulizer output was characterized by both the volume and amount of drug nebulized over time. A time-of-flight aerosol spectrometer (API Aerosizer; TSI; St. Paul, MN) was used to determine aerosol particle size and size distribution. The rate and amount of drug output that falls within the respirable fraction (RF) were compared among the devices. The RF was defined as the percentage of aerosolized particles with an MMAD of 1 to 5 µm. Quantification of drug was performed by high-performance liquid chromatography assay based on a previously published method.19

Physicochemical Properties of Solutions

Caspofungin solutions of 10 mg/mL and 30 mg/mL were analyzed for osmolality, pH, viscosity, and surface tension. All physicochemical measurements on drug solutions were performed at ambient temperature in triplicate. If necessary, solutions were adjusted using appropriate pharmaceutical buffers (ie, 0.3N NaOH to neutralize acidity) to achieve desired osmolality (150 to 550 mosm/kg), chloride ion content (31 to 300 mmol/L), and pH 7.4 within tolerable limits of the airways. Respective instruments were used to measure physicochemical properties of the solutions: osmolality (Osmette, model 5004; Precision Systems; Natick, MA), pH (Accumet, model 15; Fisher Scientific; Pittsburg, PA), viscosity (Cannon-Fensce Calibrated Viscometer; Fisher Scientific), and surface tension (Surface Tensiometer 20; Fisher Scientific).

Nebulization

The following three jet nebulizing systems were used to aerosolize drug solutions: Micromist (Hudson RCI; Temecula, CA), Sidestream MS 2400 (Invacare; Elvira, OH), and Pari LC Star (Pari Respiratory Equipment; Midlothian, VA). The first nebulizer was a disposable type for inpatient use, while the latter two were reusable types for home use. The following compressors were paired with the above nebulizers, respectively, for testing: Pulmo-Aide (model 5650D; DeVilbiss; Somerset, PA), Envoy (model IBC 1192; Invacare), and Proneb Ultra (Pari Respiratory Equipment).

The nebulizer/compressor system operating pressures and flow rates without liquid present were measured using a pressure gauge (Dwyer Capsulhelic model 4250; Dwyer Instrument Company; Michigan City, IN) and a bubble meter flow calibrator (Mini-Buck model M-30; A.P. Buck; Orlando, FL). The dry operating conditions for the respective nebulizer systems were as follows: Pulmo-Aide/Micromist, 10 pounds per square inch (psi) pressure and 6.5 L/min; Envoy/Sidestream MS 2400, 10 psi and 6.8 L/min; and Proneb Ultra/Pari LC Star, 20 psi and 3.9 L/min.
The lower flow rate in spite of the higher operating pressure for the Pari LC Star was due to the smaller orifice compared to the other two nebulizers.

A fill volume of 5 mL of drug solution was nebulized for each run. Nebulization was conducted until visual observation indicated depletion of the solution to less than approximately 1 mL remaining in the nebulizer reservoir. Nebulizer output in terms of amount of drug nebulized was measured in duplicate at ambient temperature. The amount of drug nebulized equaled the difference in the weight of drug solution present in the nebulizer reservoir before and after nebulization. In addition, the amount of drug nebulized was determined by high-performance liquid chromatography assay as the difference in the amount of drug present in the nebulizer reservoir before and after nebulization.

Particle size and distributions were characterized by the API Aerosizer time-of-flight aerosol spectrometer. Aerosolized drug was conducted into a flow-through chamber of approximately 8 L in volume. Samples were drawn from the chamber at 2 L/min into the aerosol spectrometer. A 2-min sample was taken at the beginning and the end of each trial. Although capable of measuring particles at a rate of up to $10^5$/s, the experimental conditions were chosen to limit the count rate to < $10^4$/s to avoid coincidence errors. The resulting data were analyzed, displayed, and archived to an attached computer. Results included a graphic plot of particle size distribution, calculated MMAD and geometric SD, and tabulated data showing the fraction of aerosol in the respirable size range of 1 to 5 μm aerodynamic diameter. Calibration of the aerosol spectrometer was checked at the beginning and the end of the series of experiments using monodisperse aerosols produced from polystyrene latex microsphere reference suspensions (Duke Scientific; Palo Alto, CA) nebulized from a jet nebulizer (Collison MRE, model CN24; BGI; Waltham MA).

### Results

**Physicochemical Properties**

The physicochemical properties of caspofungin solutions of 10 mg/mL and 30 mg/mL are shown in Table 1. Unadjusted solutions were acidic with pH < 6.3. Adjustment of solutions to physiologic pH was achieved with the addition of 0.3N NaOH. When 0.9% NaCl rather than sterile water was used as the diluent, the drug solutions contained chloride ions and osmolalities in the range within tolerable limits of the airways. The viscosity of the solutions did not increase significantly with increasing concentration and was similar to that of water, 1.0 centistokes (cst) and 1.3 cst for 10 mg/mL and 30 mg/mL caspofungin solutions, respectively. The surface tension of solutions was slightly reduced at the higher concentration tested (95.6 dyne/cm vs 89.8 dyne/cm), which may indicate minimal surfactant properties that might lead to foaming during aerosolization. Both caspofungin 10 mg/mL and 30 mg/mL solutions diluted in 0.9% NaCl appeared to be comparable to solutions that contain ideal physicochemical parameters for airway tolerance. The adjusted solutions were then subjected to nebulization experiments.

**Nebulization Output and Particle Size Distribution**

The amount of drug nebulized over time and the percentage of aerosols generated within the RF were compared among three nebulizing systems using two different concentrations of adjusted caspofungin solutions (10 mg/mL vs 30 mg/mL) [Figs 1–3]. The output rate, expressed in milligrams of drug aerosolized per minute, was proportional to the concentration of the drug solution (Fig 1). Specifically, the Envoy/Sidestream MS 2400 and Pulmo-Aide/Micromist systems aerosolizing a solution at a three times greater concentration (30 mg/mL vs 10 mg/mL) delivered a three times greater amount of drug output (12.50 mg/min vs 4.03 mg/min and 14.72 mg/min vs 5.4 mg/min, respectively). However, the Proneb Ultra/Pari LC Star system produced an even greater amount of drug output with increasing concentration of caspofungin solution (11.98 mg/min vs 2.26 mg/min for 30 mg/mL and 10 mg/mL of caspofungin solutions, respectively).

With respect to the aerosol particle size, the percentage of aerosols generated that fell within the RF (MMAD 1 to 5 μm) optimal for lung deposition appeared to vary by concentration of drug solution and nebulizing systems tested (Fig 2, 3). At the lower concentration of drug solution tested, both the Pulmo-Aide/Micromist and Proneb Ultra/Pari-LC Star nebulizing systems generated ≥ 83% of particles in the RF compared to 44% with the Envoy/Sidestream MS 2400 system. When tested at the higher drug concentration (caspofungin, 30 mg/mL), the Pulmo-Aide/Micromist system yielded the lowest amount of drug droplets within the RF (35%). The Proneb Ultra/Pari-LC Star system performance was consistent and gave a high percentage of aerosols within the RF for both high and low drug concentrations (93% vs 83%).

Based on the drug output in RF, we further compared efficiency of the three nebulizing systems

### Table 1—Physicochemical Properties of Caspofungin Solutions Adjusted for pH and Chloride Ion Concentrations*

<table>
<thead>
<tr>
<th>CAS Formulations</th>
<th>Chloride Ion Concentration, mmol/L</th>
<th>pH</th>
<th>Osmolality, mosm/kg</th>
<th>Viscosity, cst</th>
<th>Surface Tension, dyne/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/mL</td>
<td>155</td>
<td>7.4</td>
<td>301</td>
<td>1.0</td>
<td>95.6</td>
</tr>
<tr>
<td>30 mg/mL</td>
<td>155</td>
<td>7.4</td>
<td>326</td>
<td>1.3</td>
<td>89.8</td>
</tr>
</tbody>
</table>

*CAS = caspofungin.*
by simulating delivery of 50 mg of caspofungin aerosols in the respirable range (Table 2). The dose was selected arbitrarily as the target for drug delivery based on the recommended dose for systemic administration. Efficiency here was measured by the least drug wastage and shortest time required to deliver the target dose in optimal range of aerosol particle size. We compared the time and amount of drug required in order to deliver the target dose within the respirable range by concentration of caspofungin solution and device. In the hospital setting where disposable nebulizers are used, the Pulmo-Aide/Micromist system appeared to be most efficient: only 59 mg of caspofungin in a 10-mg/mL concentration (5.9-mL fill volume) is required to deliver the target dose of 50 mg over 15 min. However, the Proneb Ultra/Pari LC star system is the most efficient system when reusable nebulizers are used in the home setting: only 54 mg of caspofungin in 30-mg/mL concentration (1.8-mL fill volume) is required for nebulization over 5 min to deliver the target dose.

**Table 2—Simulated Dose and Nebulization Time Required To Deliver Caspofungin, 50 mg, in RF Based on Device Efficiency**

<table>
<thead>
<tr>
<th>Nebulizer System</th>
<th>Compressor/Nebulizer</th>
<th>Caspofungin Formulation, mg/mL</th>
<th>Dose, mg</th>
<th>Nebulization Time/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmo-Aide/Micromist</td>
<td>10</td>
<td>59/14.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>132/10.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Envoy/Sidestream MS 2400</td>
<td>10</td>
<td>114/21.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>89/6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ProNeb Ultra/Pari LC Star</td>
<td>10</td>
<td>60/25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>54/4.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Drug delivery via inhalation of aerosols is an attractive route of administration to attain high localized drug concentrations in the lungs while minimizing systemic toxicities. In addition, nebulization of drugs (otherwise available only for IV administration) via portable nebulizing devices allows for administration of drug to patients in an ambulatory setting. Attainment of maximal efficacy from aerosolized therapy depends in part on physicochemical properties of drug solution and the nebulizing system used.17

Caspofungin, a new antifungal agent with proven efficacy for the treatment of invasive aspergillosis, has ideal potential as an aerosol for the prevention of pulmonary aspergillosis in immunocompromised hosts. While the prophylactic potential of another derivative in the same class as caspofungin has been demonstrated in a rat model of pulmonary aspergillosis,12 to our knowledge, inhaled caspofungin therapy has not been evaluated in humans. The suitability of caspofungin for aerosol administration is important to determine in vitro with respect to factors that can influence efficacy and airway toler-
nebulization time.21 A reasonable brand and flow rate, the volume of diluent has been shown to affect nebulization time. Among other factors such as nebulizer particles and efficiency of the devices used for nebulization. Increased fill volume can reduce the amount of drug trapped in the nebulizer cup but requires a longer nebulization time; however, the time of nebulization can be offset by the use of a device with higher flow rates. We arbitrarily tested caspofungin solutions in two drug concentrations, 10 mg/mL and 30 mg/mL, to allow delivery of a dose of 50 mg in volume not > 5 mL.

When evaluated for selected physicochemical properties, we found that caspofungin at both concentrations dissolves readily in solution of either water or 0.9% NaCl. Viscosity of both solutions measured close to that of water that readily formed aerosols on jet nebulization. However, caspofungin solutions were found to have acidic pH (pH 6.3), which required addition of 0.3N NaOH buffer for adjustment to physiologic range of pH 7.4. Solutions of extreme pH have been shown to affect airway tolerability by eliciting cough.23 In addition, we recommend that 0.9% NaCl be used as drug diluent for inhalation rather than sterile water in order to achieve concentrations of chloride ions and osmolalities within the range of tolerable limits of the airways.

Three commercial jet nebulizing systems were evaluated to determine the optimal delivery system in terms of output efficiency and particle size generated for the adjusted caspofungin solutions. The amount of drug aerosolized over time and the range of aerosol particle size generated have been shown to vary widely dependent on the device used.24 We found that the amount of drug output over time increases proportionally with increasing concentration for two systems (Envoy/Sidestream MS 2400 and Pulmo-Aide/Micromist) and twice the expected increase for the Pari LC Star jet nebulizer coupled with the Ultra compressor to achieve the greatest efficiency in aerosol delivery. Aerosolization is complete in < 15 min for both systems.

We recognize several potential limitations to our in vitro evaluation of the physicochemical factors involved in generating caspofungin aerosols and characterization of the aerosol particle size and distribution. Several factors can potentially affect aerosol deposition in the lungs in vivo. First, the breathing pattern of the patient can affect aerosol deposition in the airways.14 Despite the generation of a high percentage of caspofungin aerosols in the RF for certain nebulizing systems tested, rapid and shallow breathing of the patient can enhance aerosol deposition in large airways by inertial impaction. Aerosols deposited in large airways can be readily exhaled out or cleared by mucociliary action and do not reach the target site which is the peripheral airways and alveoli in the lungs.17 In addition, humidity in the airways may also increase the particle size of hygroscopic droplets once they enter the respiratory tract, although this has not been demonstrated to be a significant determinant in therapeutic aerosol deposition.17 Finally, aerosol particle size and size distribution can be determined via different methods. Instruments such as cascade impactors and multistage liquid impingers determine mass size distribution directly, whereas light-scattering aerosol photometers and the time-of-flight spectrometer used in this work infer mass size distribution from individual particle measurements and can be subject to error if the sampled aerosol concentration is too high.20,25 Therefore, we were careful to maintain experimental conditions that minimized such error sources. It should also be noted that the API Aerosizer measured droplet size distribution, and the drug content of the respirable droplet mass fraction was estimated assuming a uniform concentration of drug in all droplets. This is a reasonable assumption given the highly quasimonodisperse nature of the nebulizer aerosols.

CONCLUSION

Caspofungin in 0.9% NaCl solution after adjustment of pH appears to be suitable for aerosol drug
delivery based on its physicochemical properties, which lie within tolerable limits of the airways. Two commercial nebulizing systems efficiently aerosolize the drug, the choice of which depends on whether a disposable or reusable type of jet nebulizer is most suitable for the particular patient care setting. The therapeutic potential of aerosolized caspofungin in preventing invasive aspergillosis in immunocompromised hosts warrants further evaluation in animal and human trials.

**REFERENCES**

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