Bronchopulmonary Disposition of Micafungin in Healthy Adult Volunteers

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Invasive aspergillosis has drawn serious attention because of its high mortality and increasing prevalence within immunocompromised patients since the 1990s (13, 19, 20). Because most invasive aspergillosis infections occur in the respiratory tract (20), it is fundamentally important to provide adequate antifungal exposure to this difficult-to-reach infection site. As a result of the recent success of echinocandins combined with other first-line agents, the idea of combination therapy for invasive aspergillosis has been given serious consideration (16, 20). Micafungin, a member of the echinocandin class, has potential in vitro activity against Aspergillus species and is considered an acceptable salvage therapy option for invasive pulmonary aspergillosis (4, 22). While the pharmacokinetics of micafungin in plasma have been well described, the bronchopulmonary disposition is largely unknown, aside from data from animal studies (7). Bronchoscopy and bronchoalveolar lavage (BAL) studies with uninfected volunteers have become a useful approach for determining intrapulmonary pharmacokinetics for antimicrobials (3, 5, 6). To our knowledge, we describe the first investigation to determine the bronchopulmonary disposition of an echinocandin in healthy volunteers.

This prospective phase I pharmacokinetic study was reviewed and approved by the Hartford Hospital investigational review board. The trial included 15 nonsmoking, healthy adults (20 to 46 years old) who met eligibility requirements based on a comprehensive medical evaluation and provided written informed consent prior to the study. Included participants received three 150-mg doses of micafungin (Astellas Pharma US, Inc., Deerfield, IL) administered intravenously over 1 h every 24 h.

Blood samples were collected at 0 h (before the third dose), 1 h (at the end of the infusion of the third dose), and 4, 12, and 24 h after the third dose. As described previously, single bronchoscopy and BAL procedures for each participant were performed at either 4, 12, or 24 h after the third dose (five participants per time point) (3). After the initial aspirate was discarded, the remaining BAL aspirates were pooled (mean volume ± standard deviation, 95 ± 19 ml) and small portions were separated for complete cell count (mean value, 1.1 × 10⁷ cells/liter) and urea concentration determination. The remaining BAL fluid was centrifuged to separate the cell pellet. Urea concentrations in BAL fluid and plasma were analyzed with a colorimetric enzymatic assay (urea nitrogen diagnostic kit no. 640; Sigma, St. Louis, MO) by a detection method employing a spectrophotometer (Cary 50 series; Varian, Walnut Creek, CA). Micafungin concentrations in plasma, BAL fluid, and the lysed cell pellet were measured by a modified and validated high-pressure liquid chromatography method (8, 20) at the Fungal Testing Laboratory (San Antonio, TX). The standard curves for each of the matrices were linear over a range of 0.05 to 25.0 μg/ml (r² = 0.99), while the interday and intraday coefficients of variation were ≤11.3 and <18%, respectively. Concentrations in epithelial lining fluids (ELF) and alveolar macrophage cells (AC) were calculated using the urea dilution method as described previously (3, 5, 6). Micafungin pharmacokinetics were described by noncompartmental methods using WinNonlin (version 5.0; Pharsight Corporation, Mountain View, CA), and levels of penetration into ELF and AC were analyzed with a colorimetric enzymatic assay (urea nitrogen diagnostic kit no. 640; Sigma, St. Louis, MO) by a detection method employing a spectrophotometer (Cary 50 series; Varian, Walnut Creek, CA). Micafungin concentrations in plasma, BAL fluid, and the lysed cell pellet were measured by a modified and validated high-pressure liquid chromatography method (8, 20) at the Fungal Testing Laboratory (San Antonio, TX). The standard curves for each of the matrices were linear over a range of 0.05 to 25.0 μg/ml (r² = 0.99), while the interday and intraday coefficients of variation were ≤11.3 and <18%, respectively. Concentrations in epithelial lining fluids (ELF) and alveolar macrophage cells (AC) were calculated using the urea dilution method as described previously (3, 5, 6). Micafungin pharmacokinetics were described by noncompartmental methods using WinNonlin (version 5.0; Pharsight Corporation, Mountain View, CA), and levels of penetration into ELF and AC were

<table>
<thead>
<tr>
<th>Collection time (h)</th>
<th>Plasma Conc. (μg/ml)</th>
<th>ELF Conc. (μg/ml)</th>
<th>AC Conc. (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>14.8 ± 1.6</td>
<td>0.52 ± 0.1</td>
<td>10.4 ± 5.6</td>
</tr>
<tr>
<td>12</td>
<td>7.4 ± 1.4</td>
<td>0.44 ± 0.1</td>
<td>8.4 ± 5.5</td>
</tr>
<tr>
<td>24</td>
<td>4.8 ± 0.6</td>
<td>0.43 ± 0.2</td>
<td>14.6 ± 8.6</td>
</tr>
</tbody>
</table>

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calculated using a composite area under the curve for the 24-h dosing interval (AUC_{0-24}).

Steady-state concentrations were achieved in all participants, as reflected by the lack of difference in plasma concentrations at 0 h (4.28 ± 1.02 μg/ml) and 24 h (4.67 ± 0.96 μg/ml) after the third dose (P = 0.215 by a paired t test). The pharmacokinetics of micafungin in plasma were consistent with the findings of previous studies (8, 15): maximum concentration of the drug in plasma, 19.1 ± 5.1 μg/ml; AUC_{0-24}, 219.7 ± 37.6 μg · h/ml; volume of distribution, 15.0 ± 4.4 liters; steady-state clearance, 0.70 ± 0.13 liters/h; and half-life, 14.7 ± 3.1 h. Concentrations in plasma, ELF, and AC are presented in Table 1. These data resulted in composite AUC_{0-24} values for ELF and AC, as calculated via the trapezoidal rule, of 10.2 and 233.6 μg · h/ml, respectively. The calculated AUC for ELF/AUC for plasma and AUC for AC/AUC for plasma penetration ratios were 5 and 106%, respectively. Despite micafungin’s proving to distribute predominately intracellularly, concentrations in all matrices, including projected concentrations of free drug in plasma, were above the median minimum effective concentration (MEC) of 0.06 μg/ml for conidial strains of Aspergillus fumigatus throughout most of the dosing interval (Fig. 1) (2).

In view of these findings, the disposition of micafungin predominantly into AC rather than ELF may prove to be a favorable characteristic of the drug. When Aspergillus conidia evade ciliary clearance, AC along with neutrophils are the primary host defense (9, 11, 14). Although the exact pathogenesis is unclear, it is suggested that the invading Aspergillus extends hyphae in a direct intracellular pathway through alveolar and endothelial cells (10).

Recently, the pharmacodynamics of echinocandins in animal studies of invasive aspergillosis have been described (12, 23). These antifungals display concentration-dependent activities against Aspergillus spp., which are maximized with a peak-concentration/MEC ratio of 10. While this exposure target was derived from total concentrations in plasma, the concentrations in AC obtained throughout the dosing interval would likely achieve this end point relative to the MEC_{90} for A. fumigatus (2).

Although micafungin is a >99% protein-bound drug (1, 4), we referenced ELF and AC penetration to the total drug AUC_{0-24} in plasma for several reasons. The significance of high-level protein binding by echinocandins remains controversial (1, 17, 18). Additionally, the degree of protein binding of micafungin in ELF is currently unknown, and the alveolus is usually devoid of albumin. One limitation to this experiment is that precise peaks and times to peak concentrations of micafungin in ELF and AC could not be established since a limited number of time points were evaluated and no sampling was conducted before 4 h. For similar reasons, we were unable to fully characterize the intrapulmonary pharmacokinetics (e.g., half-life in ELF or AC). Further characterization of micafungin intrapulmonary pharmacokinetics and disposition in infected patients is warranted.

In summary, micafungin at 150 mg once daily in healthy volunteers concentrates predominately within AC, with complete (106%) penetration relative to the total plasma drug exposure. These data provide encouraging support for clinical investigations determining the role of micafungin in the treatment or prophylaxis of invasive pulmonary aspergillosis.

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REFERENCES


