Compartmentalized Intrapulmonary Pharmacokinetics of Amphotericin B and Its Lipid Formulations

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We investigated the compartmentalized intrapulmonary pharmacokinetics of amphotericin B and its lipid formulations in healthy rabbits. Cohorts of three to seven noninfected, catheterized rabbits received 1 mg of amphotericin B deoxycholate (DAMB) per kg of body weight or 5 mg of either amphotericin B colloidal dispersion (ABCD), amphotericin B lipid complex (ABLC), or liposomal amphotericin B (LAMB) per kg once daily for a total of 8 days. Following sparse serial plasma sampling, rabbits were sacrificed 24 h after the last dose, and epithelial lining fluid (ELF), pulmonary alveolar macrophages (PAM), and lung tissue were obtained. Pharmacokinetic parameters in plasma were derived by model-independent techniques, and concentrations in ELF and PAM were calculated based on the urea dilution method and macrophage cell volume, respectively. Mean amphotericin B concentrations ± standard deviations (SD) in lung tissue and PAM were highest in ABLC-treated animals, exceeding concurrent plasma levels by 70- and 375-fold, respectively (in lung tissue, 16.24 ± 1.62 versus 2.71 ± 1.22, 6.29 ± 1.17, and 6.32 ± 0.57 μg/g for DAMB-, ABCD-, and LAMB-treated animals, respectively [P = 0.0029]; in PAM, 89.1 ± 37.0 versus 8.92 ± 2.89, 5.43 ± 1.75, and 7.52 ± 2.50 μg/ml for DAMB-, ABCD-, and LAMB-treated animals, respectively [P = 0.0246]). By comparison, drug concentrations in ELF were much lower than those achieved in lung tissue and PAM. Among the different cohorts, the highest ELF concentrations were found in LAMB-treated animals (2.28 ± 1.43 versus 0.44 ± 0.13, 0.68 ± 0.27, and 0.90 ± 0.28 μg/ml in DAMB-, ABCD-, and ABLC-treated animals, respectively [P = 0.0070]). In conclusion, amphotericin B and its lipid formulations displayed strikingly different patterns of disposition in lungs 24 h after dosing. Whereas the disposition of ABCD was overall not fundamentally different from that of DAMB, ABLC showed prominent accumulation in lung tissue and PAM, while LAMB achieved the highest concentrations in ELF.

Invasive mycoses of the lung caused by opportunistic filamentous fungi, such as Aspergillus spp., Zygomyces, Fusarium spp., and Scedosporium spp., are frequent and often cause fatal infections in immunocompromised patients, particularly those with cancer and those who have undergone hematopoietic stem cell transplantation. Despite the emergence of novel antifungal triazoles and echinocandin lipopeptides, amphotericin B and its lipid formulations remain important options for the treatment of probable and proven infections (13).

The four licensed formulations of amphotericin B have different physicochemical characteristics that result in distinct plasma pharmacokinetics (14, 27, 30). However, whether and to what extent these different dispositions of amphotericin B in plasma lead to different disposition patterns in distinct compartments of the lung are largely unknown.

The purposes of the present experimental studies, therefore, were to investigate the comparative intrapulmonary dispositions of the four licensed amphotericin B formulations in epithelial lining fluid (ELF), pulmonary alveolar macrophages (PAM), and total lung tissue and to relate these concentration data to their disposition in peripheral blood monocytes (PBM) and plasma.

MATERIALS AND METHODS

Study drug. Deoxycholate amphotericin B (DAMB) was prepared from commercially available amphotericin B (Fungizone; Bristol-Myers Squibb, Princeton, NJ); the initial powder was dissolved with sterile water and then further diluted with 5% dextrose in water, as recommended, to a final concentration of 1 mg/ml. Amphotericin B colloidal dispersion (ABCD) (Amphotec; Intermune, Brisbane, CA) was provided as lyophilized sterile powder (100 mg/vial). Prior to its use, the powder was dissolved in 20 ml of sterile water and then further diluted with 5% dextrose in water to a final concentration of 1 mg/ml. Amphotericin B lipid complex (ABLC) (Abelcet; Enzon Pharmaceuticals, Bridgewater, NJ) was provided as a 5-mg/ml solution in 20-ml vials and further diluted to a 1-mg/ml solution with 5% dextrose in water prior to use. Liposomal amphotericin B (LAMB) (AmBisome; Fujisawa USA, Deerfield, IL) was prepared from lyophilized powder. The powder was initially reconstituted with sterile water, shaken vigorously for 30 seconds, filtered through a 5-μm filter, and further diluted with 5% dextrose in water to a final concentration of 1 mg/ml. All drugs were freshly prepared prior to use.

Animals. Healthy female New Zealand White rabbits (Oryctolagus cuniculus; Hazleton, Denver, CO) weighing 2.5 to 3.5 kg were used in all experiments. They were individually housed and maintained with water and standard autoclaved rabbit feed ad libitum according to National Institutes of Health (NIH) Guide-
lines for Laboratory Animal Care (18). Vascular access was established in each rabbit prior to experimentation by the surgical placement of a subcutaneous silastic central venous catheter under general anesthesia, as previously described (29).

Experimental design and dosage selection. Five groups of three to seven rabbits each were studied. Animals received either 1 mg/kg of body weight/day DAMB, 5 mg/kg/day ABCD, 5 mg/kg/day ABCL, 5 mg/kg/day LABM, or 5% glucose in water over 5 min by a steady intravenous bolus once daily for a total of 8 days. On day 9 of the experiment, 24 h after the last dosage, rabbits were sacrificed by intravenously administered pentobarbital, following a blood drawing to harvest peripheral blood monocytes and to determine the urea nitrogen level in plasma. Immediately after the rabbits were sacrificed, postmortem examinations were performed, and lungs and intact tracheobronchial trees were aseptically removed.

Dosage selections for ABCD, ABCL, and LAMB were based on the results of previous infection models in rabbits, which have demonstrated equivalency to standard dosages of DAMB at 5 but not at 1 mg/kg/day (1, 10). These dosages also represent the standard dosages approved by the U.S. Food and Drug Administration (FDA) for the treatment of invasive fungal infections in humans (2).

Plasma pharmacokinetics. Following the last dose, a minimal sampling strategy was used to obtain both the peak concentration (Cmax) and the trough concentration (Cmin) in plasma, the area under the concentration-time curve from 0 to 24 h (AUC(0–24)), the apparent volume of distribution at steady state (Vss), and the total clearance (CL) rate. This approach allowed for the direct determination of pharmacokinetic parameters in each animal while minimizing perturbation of the experiment caused by excessive blood loss. Time points for minimal plasma sampling were determined from full plasma concentration-time profiles of healthy rabbits. Plasma sampled immediately before and after administration of the drug and then at 1, 6, 12, and 24 h postdosing was found to reflect full plasma concentration-time profiles of all four formulations (12).

BAL. Lungs and intact bronchoalveolar trees were carefully dissected and aseptically removed in toto for bronchoalveolar lavage (BAL) and harvest of PAM and lung tissue. BAL was performed on each lung preparation by the instillation and subsequent aspiration of 10 ml of sterile normal saline two times into the clamped trachea with a sterile 12-ml syringe. The aspirated volume was recorded, and specimens were centrifuged immediately for 10 min at 2,000 × g. The supernatant was separated from the cell pellet and stored at −80°C until assayed.

Harvest of pulmonary alveolar macrophages and lung tissue. Following bronchoalveolar lavage, the edges of the pulmonary lobes were cut and 20 ml of phosphate-buffered saline was forcefully injected into each lobe three to four times per lobe (24). The lavage washings, rich in pulmonary alveolar macrophages, were collected, filtered through sterile gauze pads, and pelleted at 400 × g at 4°C for 10 min. The supernatant was removed, and the pellets were resuspended in Hank's balanced salt solution free of Ca2+ and Mg2+. Contaminating erythrocytes were lysed by hypotonic buffered saline. More than 95% of the purified cells were monocytes and macrophages by morphology and nonspecific esterase staining. The yield of PAM from each rabbit ranged from 35 to 106 to 90 × 106 cells. Pulmonary alveolar macrophages were sonicated three times for 20 s each with a sonic Dismembrator (Artect Systems Corp., Farmingdale, NY) set at the 35% cycle; the micropor sonic transducer was used, and samples were placed on ice and then stored at −80°C until assayed. After the completion of the pulmonary alveolar tissue harvest, the remaining buffer solution was blotted from lung tissue with Micro Wipes (Scott Paper Company, Philadelphia, PA), and lung tissue was stored at −80°C until assayed.

Harvest of peripheral blood monocytes. Thirty milliliters of blood was obtained from each animal immediately prior to sacrifice. PBM were separated from blood buffy coats by centrifugation over Ficoll as previously described (23). Cells were resuspended in Hank's balanced salt solution free of Ca2+ and Mg2+ and counted. The yield of PBM from each rabbit ranged from 35 × 106 to 160 × 106 cells. PBM were sonicated three times for 20 s each with a sonic Dismembrator (Artect Systems Corp., Farmingdale, NY) set at the 35% cycle; the micropor sonic transducer was used, and samples were placed on ice and then stored at −80°C until assayed.

Determination of ELF volumes and concentrations of amphotericin B in ELF. As urea diffuses freely throughout body fluids, the volume of ELF can be estimated from the dilution of urea in BAL fluid (22). The concentrations of urea in sera were analyzed by the Clinical Chemistry Laboratory of the Clinical Center of the NIH by a coupled urease-glutamate dehydrogenase method modified by Roche Diagnostics (Indianapolis, IN). Urea in the BAL fluid supernatant was measured by a modified enzymatic assay (blood urea nitrogen kit 640A; Sigma, St. Louis, MO) read with an Ultraspec 2000 spectrophotometer (Pharmacia Biotech, Cambridge, United Kingdom). The proportions of reagent to specimen were changed from 3.0 ml/0.005 ml to 2.5 ml/0.5 ml, as recommended by the manufacturer, as reported by Renard et al. (22). A standard curve for normal saline ranging from 0.046 to 1.5 μl was used and was linear (r2 = 0.999). Accuracy and precision were within 10%, as assessed by separately prepared quality control samples (0.093 and 0.750 μg/ml, respectively), run in triplicate with standard curve and supernatants.

The volume of ELF in BAL fluid was derived from the following relationship: VElf = VELF × (ureaBAL/ureaSerum), where VElf is the volume of ELF sampled by BAL, VELF is the volume of aspired BAL fluid, and ureaSerum is the concentration of urea in serum.

The concentration of amphotericin B in epithelial lining fluid (CElf) was derived by the following equation: CElf = CRBAL × (VElf/VBAL), where CRBAL is the measured amphotericin B concentration in the BAL fluid supernatant (9).

Determination of concentrations of amphotericin B in PAM and PBM. The total volumes of PAM and PBM were determined from the absolute cell counts by assuming a mean monocyte/macrophage cell volume of 2.42 μm106 cells (5). The concentrations of amphotericin B in PAM and PBM (C PAM and CPBM, respectively) was derived by the following equation: CPAM or CPBM = (AMBel/VCel), where AMBal is the amphotericin B concentration in 1 ml of suspension, and VCel is the volume of pulmonary alveolar macrophages in the 1-ml suspension (9).

Processing and preparation of analytical samples. Blood samples for analytical purposes were collected in heparinized syringes, and plasma was separated by centrifugation. Like the BAL fluid, pulmonary alveolar macrophage, lung tissue, and plasma samples were stored at −80°C until assayed.

Extraction of amphotericin B was achieved by using a 1:2 (vol/vol) ratio of methanol to plasma and a 1:1 (vol/vol) ratio of methanol to BAL fluid or pulmonary alveolar macrophages. This was followed by incubation for 30 min at 4°C, centrifugation at 2,000 × g for 10 min, transfer of the methanolic supernatant into a 0.22-μm Durapore filter tube, and centrifugation at 4,000 × g for 4 min for injection. Standards and quality controls were prepared similarly from pooled normal rabbit serum (Gibco Laboratories, Grand Island, NY) or Hanks’ balanced salt solution (Gibco Laboratories, Grand Island, NY) or Hanks’ balanced salt solution (Gibco Laboratories, Grand Island, NY) or Hanks’ balanced salt solution (Gibco Laboratories, Grand Island, NY). For the extraction of amphotericin B from lung tissue, an aliquot of approximately 1 g of tissue was homogenized directly with ice-cold methanol (1:2 [wt/vl] in a high-speed tissue homogenizer (Tissue-Tek, Tekmar, Cincinnati, OH) two times for 20 s each. Homogenized lung tissue samples were incubated for 30 min at 4°C and centrifuged at 2,000 × g for 10 min. The methanolic supernatant was transferred into a 0.22-μm Durapore filter tube and centrifuged at 4,000 × g for 4 min for injection. Standards and quality control samples were prepared similarly by adding known amounts of amphotericin B to drug-free lung homogenates. Amphotericin B concentrations in lung tissue were calculated to 1 g of tissue. For all matrices, blank samples also were assayed to ensure the absence of interfering peaks.

Analytical method. Concentrations of all four investigated formulations were determined as concentrations of amphotericin B by an internally validated, modified reversed-phase high-performance liquid chromatographic method (12). Commercially available amphotericin B powder (Sigma, St. Louis, MO) was used as a reference standard.

The mobile phase was methanol–acetonitrile–0.0025 M Na-EDTA (500:350: 200), delivered at 1.6 ml/min. The injection volume ranged from 100 μl (plasma and lung homogenates) to 200 μl (BAL fluid and pulmonary alveolar macrophage tissues). Amphotericin B was eluted at a rate of 3.5 to 4.5 min using a C18 analytical column (Waters, Milford, MA) preceded by a NewGuard C18 prepocolumn (Perkin Elmer, Norwalk, CT) and was detected at 382 nm by UV absorbance.

Quantitation was based on the peak-height concentration response of the external calibration standard. Eight- to 12-point standard curves (0 to 5.0 μg/ml and 0 to 50 μg/ml for plasma and 0 to 10 μg/ml for lung homogenate and Hanks’ balanced salt solution) were linear, with R2 values greater than 0.999. The lower limits of quantitation were 0.040 μg/ml for plasma, 0.12 μg/g for lung tissue, and 0.010 μg/ml for Hanks’ balanced salt solution. accuracies were within ±10%, and intra- and interday variability values (precision) were <7.0% for all matrices.

Pharmacokinetic parameters. Peak plasma concentrations (Cmax) and concentrations 24 h after dosing (Cmin) were obtained directly from plasma concentration-time profiles. The plasma AUC(0–τ), the apparent Vss, and the plasma CLr were determined using standard experimental techniques (11). Pharmacokinetic parameters included plasma Cmax, AUC(0–τ), CLr, CLf, CLF, CPAM, and concentration in lung tissue (Clung) 24 h after dosing.

Statistical considerations. All values are presented as the means ± standard deviations (SD) from three to seven animals. Differences between the means of
continuous data were evaluated by Kruskal-Wallis nonparametric analysis of variance (ANOVA) with Dunn’s correction for multiple comparisons, as appropriate. A two-tailed \( P \) value of \( \leq 0.05 \) was considered statistically significant.

RESULTS

Plasma pharmacokinetics. The observed plasma concentration-time profiles of amphotericin B following once daily administration of either DAMB, ABCD, ABLC, or LAMB over 8 days are shown in Fig. 1, and the corresponding mean non-compartmental pharmacokinetic parameters are listed in Table 1. A comparison of the dispositions of amphotericin B in plasma revealed marked differences among the four compounds. Repeat dosing with 5 mg/kg of ABCD achieved peak and trough concentrations and AUC\(_{0–24}\) values similar to those of DAMB administered at the standard dosage of 1 mg/kg; the mean clearance rate of ABCD was faster and the mean \( V_{ss} \) was larger than those of DAMB. ABLC administered at 5 mg/kg was more rapidly eliminated from plasma and was associated with a larger \( V_{ss} \) and lower peak and trough concentrations as well as lower AUC\(_{0–24}\) values than those of both DAMB and ABCD. In contrast to dosing with DAMB, ABCD, and ABLC, dosing with LAMB at 5 mg/kg resulted in a prolonged circulation of amphotericin B in plasma, with slow elimination and escalated peak and trough concentrations and AUC\(_{0–24}\) values.

Intrapulmonary disposition. Mean concentrations of amphotericin B at 24 h following the last of eight daily dosages of either DAMB, ABCD, ABLC, or LAMB in lung tissue, ELF, PAM, and, for comparison, PBMs and concurrent plasma samples are listed in Table 2. Concentrations of amphotericin B in lung tissue and PAM were highest in the ABLC-treated animals. Drug concentrations in ELF were generally much lower than those achieved in lung tissue and PAM. Among the different cohorts, the highest absolute ELF concentrations were found in LAMB-treated animals (Table 2).

The mean site-to-plasma concentration ratios of amphotericin B at 24 h following the last of eight daily dosages are depicted in Fig. 2. Amphotericin B concentrations in lung tissue and PAM exceeded concurrent plasma levels severalfold in DAMB-, ABCD-, and ABLC-treated animals; the largest accumulation in these sites was found in the cohort of ABLC-treated animals, with mean (±SD) site-to-plasma ratios of 70 ± 20 and 375 ± 124, respectively.

![FIG. 1. Concentration-time profiles of total amphotericin B in plasma following once-daily dosing with either DAMB, ABCD, ABLC, or LAMB for 8 days. Each point plots the mean concentration ± SD from three to seven rabbits at the indicated time.](http://aac.asm.org/)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Mean ( C_{\text{max}} ) ± SD (μg/ml)</th>
<th>Mean ( C_{\text{min}} ) ± SD (μg/ml)</th>
<th>Mean AUC(_{0–24}) ± SD (μg · h/ml)</th>
<th>Mean ( V_{ss} ) ± SD (liters/kg)</th>
<th>Mean ( CL_t ) ± SD (liters/kg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAMB</td>
<td>1</td>
<td>5.36 ± 0.82</td>
<td>0.34 ± 0.07</td>
<td>17.4 ± 1.21</td>
<td>0.81 ± 0.10</td>
<td>0.041 ± 0.00</td>
</tr>
<tr>
<td>ABCD</td>
<td>5</td>
<td>7.66 ± 4.51</td>
<td>0.37 ± 0.12</td>
<td>20.4 ± 4.10</td>
<td>3.61 ± 0.64</td>
<td>0.187 ± 0.04</td>
</tr>
<tr>
<td>ABLC</td>
<td>5</td>
<td>1.58 ± 0.32</td>
<td>0.24 ± 0.08</td>
<td>11.8 ± 3.09</td>
<td>6.35 ± 1.25</td>
<td>0.315 ± 0.11</td>
</tr>
<tr>
<td>LAMB</td>
<td>5</td>
<td>46.7 ± 2.81</td>
<td>26.4 ± 4.99</td>
<td>887 ± 59</td>
<td>0.10 ± 0.01</td>
<td>0.001 ± 0.00</td>
</tr>
</tbody>
</table>

*All values represent the means ± SD from three to seven rabbits in each dosage group. Pharmacokinetic parameters were calculated on the basis of total amphotericin B concentrations. \( P \) values from comparisons across dosage groups by Kruskal-Wallis nonparametric ANOVA were as follows: \( C_{\text{max}} \) \( P = 0.0047 \), \( C_{\text{min}} \) \( P = 0.0014 \); AUC\(_{0–24}\) \( P = 0.0036 \); \( V_{ss} \) \( P = 0.0014 \); and \( CL_t \) \( P = 0.0014 \). Between-group comparisons using Dunn’s correction for multiple comparisons revealed significant differences (\( P < 0.05 \) to \( < 0.01 \)) between ABLC- and LAMB-treated animals for all parameters and significant differences (\( P < 0.05 \)) between ABCD- and LAMB-treated animals with respect to \( V_{ss} \) and \( CL_t \), respectively.
site-to-plasma ratios of amphotericin B in lung tissue and PAM in LAMB-treated animals were well below 1. Mean concentrations of amphotericin in ELF were similar or slightly higher than concurrent plasma levels in DAMB-, ABCD-, and ABLC-treated animals but approximately 10-fold lower in LAMB-treated animals.

**Disposition in peripheral blood monocytes.** Mean concentrations of amphotericin B associated with PBM appeared to be highest in the ABCD-treated animals and lowest in the ABLC-treated animals (Table 2). Concentrations of amphotericin B associated with PBMs were generally severalfold lower than those associated with PAM, most notably in ABLC-treated animals. However, with the exception of LAMB-treated animals, comparisons with concurrent plasma concentration values were indicative of accumulation in or with PBM (mean site-to-plasma ratios ± standard errors of the means, 3.21 ± 1.70, 9.21 ± 2.95, 4.19 ± 3.80, and 0.06 ± 0.03 for DAMB-, ABCD-, ABLC-, and LAMB-treated animals, respectively [P = 0.0387 by ANOVA]).

**DISCUSSION**

The results of these experimental pharmacokinetic studies demonstrate that therapeutic dosages of the four licensed amphotericin B formulations display markedly different plasma pharmacokinetics that, in turn, are associated with distinct disposition patterns in the lungs after multiple dosing. While the disposition of ABCD in plasma was similar to that of DAMB, ABLC was rapidly eliminated from the bloodstream and achieved comparatively lower plasma C_{max} and AUC_{0–24} values. In contrast, LAMB showed a prolonged circulation in blood, with slow elimination and escalating C_{max} and AUC_{0–24} values. Twenty-four hours after the last of eight daily dosages, mean amphotericin B concentrations in lung tissue and PAM were highest in ABLC-treated animals, exceeding concurrent plasma levels 70- and 375-fold, respectively. Mean drug concentrations in ELF were generally much lower than those achieved in lung tissue and PAM; among the different cohorts, the highest ELF concentrations were found in LAMB-treated animals. There was no apparent direct correlation between plasma and intrapulmonary drug concentrations, which is indicative of the pivotal importance of the carrier for the intrapulmonary disposition of amphotericin B.

As plasma concentrations may not necessarily predict effective concentrations at the target site, tissue penetration studies have become an integral part of the pharmacokinetic assessment of antimicrobial agents. For example, in the setting of community-acquired respiratory infections, compartmental intrapulmonary distributions and their relationship to MICs provide the scientific rationale for the use and dosing regimens of macrolides, azalides, and quinolones (3, 19). We therefore sought to further understand the distribution of amphotericin B in the lung by analyzing its distribution into PAM, ELF, and total lung tissue.

The four licensed amphotericin B formulations possess distinct physicochemical and pharmacokinetic properties. As an amphiphilic molecule that lacks significant metabolism and rapid elimination from the body, amphotericin B is preferentially taken up by cells of the mononuclear phagocytic system (7). Amphotericin B administered as ABCD and ABLC is rapidly taken up into the mononuclear phagocytic system, which is reflected by lower peak plasma concentrations and AUC values and a larger volume of distribution than those of DAMB. In contrast, amphotericin B administered as LAMB
has a prolonged circulation in the bloodstream, with strikingly high peak plasma concentrations and AUC values (6, 14, 28).

Whether and how these distinct physicochemical and pharmacokinetic features translate into different pharmacodynamic properties in the lungs are largely unknown. In single- and multidose distribution studies of rodents that used equimolar dosages of 1 mg/kg of the parent drug, concentrations in whole-lung homogenates achieved with ABCD and LAMB were lower and those achieved with ABLC were similar or slightly higher than those achieved with DAMB as the reference agent. After multiple dosing of 5-fold (ABCD and LAMB)- to 10-fold (ABLC)-higher dosages, drug accumulation in the lungs clearly exceeded that achieved by 1 mg/kg of DAMB (20, 21, 28). These differences in lung distribution appear to correspond to the results of comparative pulmonary infection models, where the lipid formulations required at least fivefold-higher dosages to produce a reduction of the residual fungal burden in lung tissue equivalent or superior to that produced by standard dosages of DAMB (1, 8, 10).

The carrier effects of the different amphotericin B formulations suggest that compartmental lung tissue concentrations may indeed be the more relevant pharmacokinetic parameters to be correlated with antifungal efficacy. This question, however, has been addressed only in very select aspects. Koizumi and coworkers investigated the distribution of amphotericin B in lung lymph following intravenous administration of 1 mg/kg of DAMB to adult sheep with lung lymph fistulas. While the lung lymph-to-plasma ratio of amphotericin B increased gradually during infusion and was more than 1 after the end of the infusion, the AUC₀₋₂₄ of plasma and lung lymph were virtually identical, suggesting that amphotericin B could easily move from plasma to the pulmonary interstitium and/or the lung lymph circulation (16). In spontaneously breathing, anesthetized cats, Matot and Pizov studied the single-dose uptake of 1 mg/kg DAMB, ABLC, and LAMB by intact lungs using double-indicator outflow curves. While the mean rates of first-pass uptake of all three formulations were similar, at 69 to 82%, ABLC exhibited prolonged retention in the lungs: 23 and 15% of the injected dose remained in the lungs for 1 and 6 h after administration, respectively, compared to ≤2% after 1 h in DAMB- and LAMB-treated animals (17). This prolonged retention of ABLC in lung tissue is consistent with our findings of highly elevated concentrations predominantly in PAM.

The data reported herein provide the first evidence for a distinct intrapulmonary disposition pattern of currently licensed amphotericin B formulations. As the plasma pharmacokinetics of all four formulations in the rabbit are similar to those in humans (12) and since fundamental structural or functional differences of the anatomical barriers between blood and airspace do not exist between the two species, the results are principally suitable for inference to patients.

The anatomic sites of airborne pulmonary mold infections are primarily extravascular and include the distal Airways and the alveoli. PAM constitute an important additional compartment, as they are abundant and constitute the first line of phagocytic host defense (25). Conceptually, pulmonary mold infections would thus require the delivery of a bioactive drug in therapeutic concentrations to these extravascular sites of infection and intracellular compartments of pulmonary macrophages.

While the exact mechanisms of drug penetration of amphotericin into the lungs remain unclear, because of their size, the carriers themselves are unlikely to pass through the nonfenestrated capillary endothelium, the capillary basement membrane, the alveolar basement membrane, and alveolar pneumocytes into the alveolar space. Instead, amphotericin B may leave the pulmonary microcirculation and enter the alveolar space by several different mechanisms: diffusion of free drug along a concentration gradient (permeation), uptake of bound and unbound drug from blood into PAM with subsequent migration into the alveolar compartment or infected tissue, uptake of lipoprotein-bound amphotericin B via endothelial lipoprotein receptors, and pinocytosis of amphotericin B by endothelial cells (15, 19, 26, 31). Whereas additional drug disposition may result from the leakage of bound and free drug into tissue when anatomic barriers are damaged by infection, inflammation, or necrosis (15, 19, 26), these proposed mechanisms do not fully account for the capacity of these large molecules to traverse the complex cellular and connective tissue barriers that separate pulmonary capillaries and alveolar spaces, particularly in noninfected lung tissue.

The differential intrapulmonary dispositions and the lack of a direct correlation between plasma- and intrapulmonary drug concentrations in this study are indicative of the pivotal importance of the carrier for the transfer of amphotericin B into different compartments of healthy lungs. At their respective therapeutic dosages, the two small, variably stable micellar formulations, DAMB and ABCD, displayed similar plasma pharmacokinetics and similar intrapulmonary disposition patterns, with considerable drug disposition into PAM and lung tissue, but with comparatively low drug concentrations in ELF. The small, stable unilamellar liposomal formulation, despite producing a severalfold increase in drug exposure in the bloodstream during the dosing interval, achieved drug concentrations in PAM and lung tissue similar to those of DAMB and ABCD but the highest ELF concentrations among all compounds, which may be explained by the relatively higher drug concentration gradient across the barrier between blood and alveolar space. ABLC, in contrast, preferentially distributed to PAM; it is conceivable that the comparatively high lung tissue concentrations are caused by the fraction of PAM that remained tissue bound after the lavage procedure. Consistent with the observations of Matot and Pizov (17), the large ribbon-like phospholipids may be trapped in the capillary bed of the lung and amphotericin B may be phagocytosed either with its carrier or following its degradation by phospholipids. The prolonged retention of ABLC in the pulmonary capillaries may thus permit a greater duration of time for uptake by PAM.

It is unknown, nevertheless, whether achieving a concentration equilibrium among PAM, lung tissue, and ELF or preferential dispositions in PAM and lung tissue similar to those of DAMB and ABCD but the highest ELF concentrations among all compounds, which may be explained by the relatively higher drug concentration gradient across the barrier between blood and alveolar space. ABLC, in contrast, preferentially distributed to PAM; it is conceivable that the comparatively high lung tissue concentrations are caused by the fraction of PAM that remained tissue bound after the lavage procedure. Consistent with the observations of Matot and Pizov (17), the large ribbon-like phospholipids may be trapped in the capillary bed of the lung and amphotericin B may be phagocytosed either with its carrier or following its degradation by phospholipids. The prolonged retention of ABLC in the pulmonary capillaries may thus permit a greater duration of time for uptake by PAM.

It is unknown, nevertheless, whether achieving a concentration equilibrium among PAM, lung tissue, and ELF or preferential dispositions in PAM and lung tissue is of therapeutic advantage. In a recent comparative pharmacokinetic/pharmacodynamic study of neutropenic mice intravenously infected with *Candida albicans* (4), amphotericin B concentrations in the lungs were highest following ABLC administration, and these enhanced concentrations correlated with enhanced potency at this site, as expressed by the 75% effective dose. However, findings from a disseminated blood-borne yeast infection may not be translated directly to the setting of airborne...
pulmonary mold infections that follow different patterns of infection and lung tissue damage.

In conclusion, therapeutic dosages of the four currently approved amphotericin B formulations displayed markedly different plasma pharmacokinetics that were associated with distinct disposition patterns in the lung after multiple doses. Whereas the intrapulmonary disposition of ABCD was overall not fundamentally different from that of DAMB, ABLC showed prominent accumulation in lung tissue, and PAM and LAMB achieved the highest concentrations in ELF.

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REFERENCES