Comparative Efficacy and Distribution of Lipid Formulations of Amphotericin B in Experimental Candida albicans Infection of the Central Nervous System

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The Journal of Infectious Diseases 2000; 182:274±82

The central nervous system (CNS) distribution and antifungal efficacy of all 4 approved formulations of amphotericin B (AmB) were investigated in a rabbit model of hematogenous Candida albicans meningoencephalitis. Treatment with AmB deoxycholate (1 mg/kg/day) or liposomal AmB (5 mg/kg/day) yielded the highest peak plasma concentration (C_max), area under concentration versus time curve from zero to 24 h (AUC_0-24), and time during dosing level > minimum inhibitory complex (MIC) values and led to complete eradication of C. albicans from brain tissue (P < .05 vs. untreated controls). By comparison, AmB colloidal dispersion and AmB lipid complex (5 mg/kg/day each) were only partially effective (not significant vs. untreated controls). There was a strong correlation of C_max, AUC_0-24, C_max/MIC, AUC_0-24/MIC, and T > MIC with clearance of C. albicans from brain tissue (P < .0002). Although pharmacodynamic parameters derived from the MIC of free AmB were highly predictive of antifungal efficacy, parameters derived from MICs of individual formulations were not predictive. AmB deoxycholate and liposomal AmB had the greatest antifungal efficacy. This activity was concentration and time dependent.

Involvement of the central nervous system (CNS) is frequent in both opportunistic and endemic mycoses [1-4]. In pediatric patients, Candida meningoencephalitis is a particularly important nosocomial fungal infection [5-10]. Despite its broad spectrum of antifungal activity, standard medical treatment with amphotericin B (AmB) deoxycholate is often limited by drug-related toxicity [11]. The lipid formulations of AmB may offer new therapeutic alternatives because of the possible delivery of higher doses of the parent compound and different distribution patterns in plasma and tissues [12-15]. However, the CNS distribution of the lipid formulations and that of AmB deoxycholate have not been investigated in a comparative fashion. Furthermore, no study has addressed the clinically important relationships of plasma concentrations, penetration into the CNS, and antifungal efficacy.

We therefore investigated the relationship between antifungal efficacy and drug concentrations of conventional AmB and its 3 novel, US Food and Drug Administration (FDA)-approved lipid formulations in a rabbit model of CNS candidiasis. This model aimed at developing new therapeutic approaches for CNS candidiasis and at providing a scientific rationale for treatment of other CNS fungal infections. We initially studied the CNS disposition of the 4 compounds after administration over 7 days at standard doses in normal, noninfected rabbits. We then established a model of subacute disseminated candidiasis that reliably produced hematogenous Candida meningoencephalitis in order to examine the distribution of all 4 formulations into the CNS of infected rabbits and their ability to clear the CNS following a 7-day course of antifungal treatment.

Materials and Methods

Drugs. AmB deoxycholate was prepared from commercial 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. AmB colloidal dispersion (Amphotec; SEQUS Pharmaceuticals, Menlo Park, CA) was provided as lyophilized sterile powder (100 mg/vial). Prior to 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. AmB lipid complex (Abelec; Liposome, Princeton, NJ) was provided as 5-mg/mL solution in 20-mL vials and further diluted to a 1-mg/mL solution with 5% dextrose in water before use. Liposomal AmB (Ambisome; Fujisawa USA, Deerfield, IL) was prepared from lyophilized powder. The powder was initially reconstituted with 12 mL of sterile water to yield a preparation containing 4 mg/mL AmB. The so-
lution was then heated to 60°C for 10 min to ensure dissolution, filtered through a 5-μm filter, and further diluted with 5% dextrose in water to a final concentration of 2 mg/mL. All drugs were freshly prepared before use.

**Animals.** We used female New Zealand White rabbits (Hazleton, Denver, PA) weighing 2.5–3.5 kg for the study. They were housed individually and maintained with water and standard autoclaved rabbit feed according to National Institutes of Health (NIH) Guidelines for Laboratory Animal Care [16]. Prior to the start of the experiments, a silastic central venous catheter was surgically placed under general anesthesia in each animal, as described elsewhere [17], to allow for repeated nontraumatic venous access.

**Distribution in noninfected rabbits.** Four groups of 4 or 5 rabbits were studied. Animals received either AmB deoxycholate (1 mg/kg/day), AmB colloidal dispersion (5 mg/kg/day), AmB lipid complex (5 mg/kg/day), or liposomal AmB (5 mg/kg/day) at 0.4 mg/min (AmB deoxycholate) and 1.2 mg/min (AmB colloidal dispersion, AmB lipid complex, and liposomal AmB) by a steady intravenous (iv) bolus once a day after normal saline loading (10 mL/kg over 5 min), for 7 doses in total. Animals were killed 30 min after dose 7 by iv pentobarbital, and blood, cerebrospinal fluid (CSF), and brain tissue were simultaneously obtained for determination of AmB concentrations.

Selection of the dosages of the lipid formulations was based on result of previous infection models in the rabbit, which have demonstrated equivalency to standard dosages of AmB deoxycholate at 5 but not at 1 mg/kg/day [18–20]. These dosages also represent standard dosages approved by the US FDA for the treatment of invasive fungal infections in humans [21].

**Distribution in infected rabbits and assessment of antifungal activity.** In order to obtain a standardized comparison of drug concentrations in infected versus noninfected animals after a minimum period of drug accumulation and to correlate this information with microbiologic data, the requirements for the design of the infection model were 2-fold: first, to reliably establish CNS involvement at the start of antifungal chemotherapy; and second, to allow for survival of animals treated with standard dosages of AmB deoxycholate for 7 days. Pilot studies revealed that an iv inoculum of 10^5 colony-forming units (cfu) of *Candida albicans* did not result in CNS infection, even in untreated animals; an inoculum of 10^6 cfu, on the other hand, led to early infectious deaths despite treatment, and an inoculum of 10^7 was precipitously lethal within 48 h. However, the iv administration of 10^6 cfu resulted in consistent infection of brain tissue at 48 h after inoculation and survival of AmB deoxycholate-treated and -untreated animals until the end of the experiment; this level was therefore selected.

Five groups of 4 or 5 rabbits each were studied in the experiments of antifungal efficacy. On day 1 of the experiment, all animals were challenged with an iv inoculum of 10^6 cfu of *C. albicans*. At 48 h after inoculation, rabbits received either AmB deoxycholate (1 mg/kg/day), AmB colloidal dispersion (5 mg/kg/day), AmB lipid complex (5 mg/kg/day), liposomal AmB (5 mg/kg/day), or 5% glucose in water at 0.4 mg/min (AmB deoxycholate) and 1.2 mg/min (AmB colloidal dispersion, AmB lipid complex, and liposomal AmB) by a steady iv bolus, after normal saline loading (10 mL/kg over 5 min) once daily for 7 days. Microbiologic clearance of blood was monitored daily by culture of whole blood drawn before dosing. Animals were killed 30 min after dose 7 on day 9 of the experiment by iv pentobarbital. Blood, CSF, and brain tissue were obtained for cultures and analysis of AmB concentrations. Microbiologic response to antifungal therapy was evaluated by quantitative assessment of concentration of *C. albicans* in cfu per gram in brain tissue or cfu per milliliter in CSF and blood, respectively.

**Minimal sampling strategy.** A minimal plasma sampling strategy was utilized on day 6 of treatment to obtain both peak plasma level (C_{max}) and the area under the concentration versus time curve from zero to 24 h (AUC_{0–24}). This approach allowed for direct comparison of pharmacokinetic and pharmacodynamic parameters with measures of outcome in each individual animal, while minimizing perturbation of outcome by excessive blood loss. Time points for minimal plasma sampling were determined from full plasma concentration versus time profiles of healthy rabbits.

<p>| Table 1. Concentrations of AmB cerebrospinal fluid (CSF) and brain tissue 30 min after last of 7 daily doses of AmB deoxycholate (t-AmB), AmB colloidal dispersion (ABCD), AmB lipid complex (ABLC), or liposomal AmB (l-AmB). |</p>
<table>
<thead>
<tr>
<th>Compound, no. of rabbits</th>
<th>Plasma, µg/mL</th>
<th>CSF, µg/mL</th>
<th>Tissue, µg/g</th>
<th>Tissue/plasma ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Noninfected animals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-AmB (5)</td>
<td>1.82 ± 0.07^a</td>
<td>0.023 ± 0.000</td>
<td>0.33 ± 0.03^b</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>ABCD (4)</td>
<td>0.85 ± 0.01</td>
<td>0.014 ± 0.007</td>
<td>0.19 ± 0.02</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>ABLC (4)</td>
<td>0.93 ± 0.03</td>
<td>0.022 ± 0.000</td>
<td>0.25 ± 0.02</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>l-AmB (5)</td>
<td>62.9 ± 0.99^c,d</td>
<td>0.024 ± 0.001</td>
<td>1.99 ± 0.35^d</td>
<td>0.03 ± 0.00^e</td>
</tr>
<tr>
<td><strong>Animals infected with <em>C. albicans</em></strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-AmB (4)</td>
<td>1.41 ± 0.14^a</td>
<td>0.026 ± 0.001</td>
<td>0.37 ± 0.03</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>ABCD (4)</td>
<td>0.96 ± 0.04</td>
<td>0.033 ± 0.004</td>
<td>0.51 ± 0.08</td>
<td>0.50 ± 0.11</td>
</tr>
<tr>
<td>ABLC (4)</td>
<td>0.84 ± 0.05</td>
<td>0.026 ± 0.002</td>
<td>0.35 ± 0.06</td>
<td>0.41 ± 0.06</td>
</tr>
<tr>
<td>l-AmB (4)</td>
<td>59.54 ± 0.88^d,e</td>
<td>0.031 ± 0.006</td>
<td>1.84 ± 0.12^d</td>
<td>0.03 ± 0.00^f</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SE. Although AmB was detectable in CSF of all rabbits, mean levels were below lower limit of quantitation (LLQ) of 0.040 µg/mL. LLQ refers to lowest concentration of analyte that can be quantified with acceptable accuracy and precision; it is not identical with sensitivity of assay, which is determined by peak/noise ratio of chromatogram.

^a_ P < .05 vs. ABCD, ABLC by Mann-Whitney U test.

^b_ P < .05 vs. ABCD by Mann-Whitney U test.

^c_ P < .005.

^d_ P < .05 vs. t-AmB, ABCD, ABLC by Mann-Whitney U test.

^e_ P < .01.

^f_ P < .05 for comparison among all groups by Kruskal-Wallis analysis of variance.
inoculum of 10^6 cfu was administered to each rabbit in 5 mL of
concentrations of the inoculum was adjusted by hemocytometer and
The suspension was centrifuged and resuspended twice more. The
10 min, and the pellet was resuspended in sterile normal saline.

Emmons modified Sabouraud dextrose broth (NIH Media De-
for 18 h. The inoculum suspension was centrifuged at 2000
g concentrations of AmB deoxycholate (D-AmB), AmB colloidal dispersion

Figure 1. Comparison of the distribution of 4 amphotericin B
(AmB) formulations into cerebrospinal fluid (CSF) and brain tissue in
infected vs. noninfected rabbits. Values are mean ± SE. *Not signif-
ificant (NS); †P < .05, infected vs. noninfected animals; #NS, infected
vs. noninfected animals and P < .05 for comparison with AmB con-
centrations of AmB deoxycholate (D-AmB), AmB colloidal dispersion
(ABCD), and AmB lipid complex (ABLC)–treated rabbits (Mann-
Whitney U test). L-AmB, liposomal AmB.

Plasma sampling immediately before and after administration of
drug and at 1, 6, 12, and 24 h after dosing was found to reflect
the full plasma concentration versus time profiles of all 4 formulations.

Organism and preparation of the inoculum. C. albicans isolate
NIH-8621 from a granulocytopenic patient with autopsy-confirmed
disseminated candidiasis was used for all experiments. By use of the
National Committee for Clinical Laboratory Standards (NCCLS)
microdilution method [22] with antibiotic medium 3 (NIH Media
Department, Bethesda, MD), the MICs of the isolate in vitro were 0.500 µg/mL for AmB deoxycholate, 0.125 µg/mL for
AmB colloidal dispersion, 0.066 µg/mL for AmB lipid complex,
1.00 for liposomal AmB, and 1.00 for free AmB (Sigma, St. Louis)
dissolved in dimethyl-sulfoxide as recommended elsewhere [22].

C. albicans was cultured from a frozen stock culture on Sa-
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Quantitation of C. albicans in tissue, CSF, and blood. In each
rabbits. Values are mean ± SE. *Not significant (NS); †P < .05, infected vs. noninfected animals; #NS, infected
vs. noninfected animals and P < .05 for comparison with AmB con-
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rabbits. Values are mean ± SE. *Not significant (NS); †P < .05, infected vs. noninfected animals; #NS, infected
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centrations of AmB deoxycholate (D-AmB), AmB colloidal dispersion
(ABCD), and AmB lipid complex (ABLC)–treated rabbits (Mann-
Whitney U test). L-AmB, liposomal AmB.
Table 2. Peak plasma levels and AUC$_{0-24}$ values of infected rabbits and microbiologic and clinical outcome.

<table>
<thead>
<tr>
<th>Treatment, no. of rabbits</th>
<th>$C_{\text{max}}, \mu$g/mL</th>
<th>AUC$_{0-24}$, $\mu$g/mL $\times$ h</th>
<th>Blood</th>
<th>Brain tissue, log (cfu/g)</th>
<th>Survival through day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>d-Amb (4)</td>
<td>3.87 ± 0.08$^a$</td>
<td>14.44 ± 0.36$^a$</td>
<td>None</td>
<td>None$^b$</td>
<td>4/4</td>
</tr>
<tr>
<td>ABCD (4)</td>
<td>1.79 ± 0.12$^a$</td>
<td>11.21 ± 1.06</td>
<td>None</td>
<td>1.64 ± 0.59 (3/4)</td>
<td>4/4</td>
</tr>
<tr>
<td>ABLC (4)</td>
<td>0.99 ± 0.09</td>
<td>10.03 ± 1.01</td>
<td>None</td>
<td>1.60 ± 0.63 (3/4)</td>
<td>4/4</td>
</tr>
<tr>
<td>t-Amb (4)</td>
<td>6.20 ± 1.32$^c$</td>
<td>1141 ± 33$^f$</td>
<td>None$^b$</td>
<td>None$^b$</td>
<td>4/4</td>
</tr>
<tr>
<td>None (5)</td>
<td>NA</td>
<td>NA</td>
<td>Growth in 3/5</td>
<td>3.38 ± 0.58 (5/5)$^f$</td>
<td>4/5</td>
</tr>
</tbody>
</table>

NOTE. d-Amb, AmB deoxycholate; ABCD, AmB colloidal dispersion; ABLC, AmB lipid complex; and t-Amb, liposomal AmB. $C_{\text{max}}$, peak plasma concentration; AUC$_{0-24}$, area under concentration vs. time curve, 0–24 h. Data are mean ± SE; data in parentheses (fungus burden in brain tissue): proportion of culture-positive rabbits. There was no growth in cerebrospinal fluid. NA, not applicable.

$^a$ $P < .05$ vs. ABCD and ABLC by Mann-Whitney U test.

$^b$ $P < .05$ vs. untreated controls by Kruskal-Wallis analysis of variance (ANOVA) and Dunn’s multiple comparisons test.

$^c$ $P < .05$ vs. ABLC by Mann-Whitney U test.

$^d$ $P < .005$.

$^e$ $P < .05$ vs. d-Amb, ABCD, ABLC by Mann-Whitney U test.

$^f$ $P < .01$ for comparison among all groups by Kruskal-Wallis ANOVA.

Results

AmB concentrations in noninfected animals. Concentrations of AmB in plasma, CSF, and brain tissue at 30 min after the last of 7 daily doses of AmB deoxycholate (1 mg/kg/day), AmB colloidal dispersion, AmB lipid complex, or liposomal AmB (5 mg/kg/day each) are depicted in table 1.

The highest plasma concentrations were achieved by liposomal AmB. These concentrations were significantly higher than those obtained with AmB deoxycholate, AmB colloidal dispersion, and AmB lipid complex ($P < .05$ for each comparison). Plasma concentrations after administration of AmB deoxycholate also were higher than after either AmB colloidal dispersion or AmB lipid complex ($P < .05$). There was no significant difference between the mean plasma levels of AmB colloidal dispersion and AmB lipid complex.

Drug concentrations in brain tissue were greatest after administration of liposomal AmB ($P < .05$ vs. AmB deoxycholate, AmB colloidal dispersion, and AmB lipid complex), followed by AmB deoxycholate ($P < .05$ vs. AmB colloidal dispersion). There was no statistically significant difference in tissue levels between AmB deoxycholate and AmB lipid complex or AmB colloidal dispersion and AmB lipid complex. As evident by the analysis of the tissue/plasma ratio in comparison to AmB deoxycholate, the lipid formulations did not appear to be associated with an increased relative tissue distribution; indeed, liposomal AmB demonstrated a significantly decreased tissue/plasma ratio when compared with the other formulations. Mean drug levels in CSF 30 min after dosing were detectable with all 4 formulations but at concentrations below the lower limit of quantification of the analytic assay.

AmB concentrations in infected animals. Concentrations of AmB in plasma, CSF, and brain tissue of infected animals at 30 min after the last of 7 daily doses of either AmB deoxycholate, AmB colloidal dispersion, AmB lipid complex, or liposomal AmB are shown in table 1.

Liposomal AmB-treated animals had the highest plasma levels at 30 min after dose 7 ($P < .05$ vs. AmB deoxycholate, AmB colloidal dispersion, and AmB lipid complex), followed by AmB deoxycholate ($P < .05$ vs. AmB colloidal dispersion and AmB lipid complex), and AmB colloidal dispersion (not significant [NS] vs. AmB lipid complex). Similar to the situation in uninfected animals, the highest brain-tissue levels were measured in animals treated with liposomal AmB ($P < .05$ vs. AmB deoxycholate, AmB colloidal dispersion, and AmB lipid complex). However, no statistically significant differences in tissue concentrations were observed among AmB deoxycholate, AmB colloidal dispersion, and AmB lipid complex–treated animals.
Mean concentrations of AmB in CSF were below the lower limit of quantitation in all 4 cohorts. Relatively higher mean brain-tissue levels, compared with noninfected animals, were observed in infected animals treated with AmB lipid complex (×1.4 vs. noninfected; NS) or AmB colloidal dispersion (×2.6 vs. noninfected; *P < .05). Although not statistically significant, this coincided with an increased tissue/plasma level ratio in both cohorts. In contrast, mean brain-tissue levels in the AmB deoxycholate– and liposomal AmB–treated cohorts were almost identical in infected and noninfected animals (<10% variation). When drug levels in CSF of infected animals were compared with those achieved in noninfected animals, no differences were noted (figure 1).

Minimal sampling pharmacokinetics of AmB. Peak plasma (Cmax) levels and AUC0–24 values obtained by minimal plasma sampling on day 6 of treatment are shown in table 2. Highest peak plasma levels were achieved by liposomal AmB (P < .05 vs. AmB deoxycholate, AmB colloidal dispersion, AmB lipid complex), followed by AmB deoxycholate (P < .05 vs. AmB colloidal dispersion and AmB lipid complex), and AmB colloidal dispersion (P < .05 vs. AmB lipid complex). The mean AUC0–24 of liposomal AmB exceeded those of the other 3 compounds by ≥80-fold (*P < .05), and the mean AUC0–24 of AmB deoxycholate was higher than the corresponding AUCs of AmB colloidal dispersion and AmB lipid complex (*P < .05).

Assessment of antifungal activity. The clinical and microbiologic end points of the infection model are shown in table 2. All animals treated with either AmB deoxycholate or liposomal AmB had negative cultures of brain tissue. By comparison, cultures of brain tissue were positive in all 5 untreated controls and in 3 of 4 animals each treated with either AmB colloidal dispersion or AmB lipid complex. As compared with untreated controls, only treatment with AmB deoxycholate and liposomal AmB led to a statistically significant quantitative reduction in the residual fungus burden in brain tissue (*P < .05; figure 2).

All infected animals had positive blood cultures for C. albicans at 24 and 48 h after inoculation. Fungemia cleared in 10 (62.5%) of 16 treated animals within 24 h after the start of antifungal therapy. All remaining animals converted to negative blood cultures at a median of 3 days (range, 2–5) after start of treatment. At the end of therapy, all blood cultures in treated animals were negative, whereas 3 of 5 control animals had ongoing fungemia. Despite negative blood cultures, 6 (37.5%) of 16 treated rabbits had positive brain-tissue cultures. CSF cultures were negative at autopsy in all animals, including untreated controls. All animals survived the 9 days of experiment, except for 1 untreated control rabbit that died of infection on day 7.

Analysis of pharmacokinetic/pharmacodynamic relationships. As displayed in table 2, rabbits treated with AmB deoxycholate and liposomal AmB had complete clearance of C. albicans from brain tissue and the highest Cmax and AUC0–24 values. The relationship between plasma and tissue concentrations of AmB and the fungus burden in brain tissue is shown in table 3. Spearman’s rank correlation revealed a strong inverse correlation between the residual burden of C. albicans in brain tissue and plasma concentrations of AmB as measured by Cmax, C30min, and AUC0–24. This relationship demonstrates that the higher the plasma concentrations of AmB (Cmax, C30min, or AUC0–24), the lower is the residual concentration of C. albicans in brain tissue. The negative Spearman’s rank correlation values reflect this inverse relationship between plasma AmB concentrations and C. albicans tissue concentrations.

There was a strong inverse correlation between Cmax/MIC, AUC0–24/MIC, and T > MIC and the residual fungus burden in brain tissue when the MIC value of the reference standard of AmB (i.e., 1 µg/mL) was utilized to compute these parameters (table 4). When the MIC-related pharmacodynamic parameters were investigated using the MIC values of the individual formulations, however, only weak or no significant correlations with antifungal efficacy were found.

Discussion

The lipid formulations of AmB allow for the administration of AmB with reduced nephrotoxicity and at higher doses than...
The results of our study, which investigated the CNS disposition and pharmacodynamics of the 4 currently available AmB formulations for the first time, show a positive association of both $C_{\text{max}}$ and AUC$_{0-24}$ with the disposition of AmB in brain tissue and demonstrate that $C_{\text{max}}$, AUC$_{0-24}$, and $T_{\text{max}}$ MIC directly correlate with antifungal efficacy. These data provide a pharmacodynamic rationale for the antifungal activity of AmB deoxycholate and liposomal AmB in the treatment of fungal infections of the CNS. The data also suggest that pharmacodynamic parameters utilizing the MIC of free AmB prepared according to recommendations of the NCCLS [22], and not those of the individual formulations, correlate with antifungal efficacy at this site.

Rabbit models have well established utility for assessing the penetration of antifungal compounds into the CNS [28, 29] and for investigating antifungal efficacy against disseminated candidiasis [30] and Candida meningitis [31, 32]. Our model was primarily designed to characterize the relationships between MIC and drug concentrations in plasma, CSF, and brain tissue with antifungal efficacy in the CNS in order to better understand the distribution and antifungal efficacy of the different AmB formulations in this compartment. The model reliably established CNS infection prior to the start of antifungal therapy, led to persistent brain infection without producing an unacceptable amount of clinically apparent CNS pathology, and ultimately allowed for the evaluation of the residual fungus burden and comparison with untreated animals. The hematogenous route of infection reflects the natural pathogenesis of most mycoses of the CNS, particularly that of Candida meningoencephalitis, which commonly originates from the invasion of the pathogen via the bloodstream [3, 33–35]. The finding that all animals had negative CSF cultures at autopsy is not surprising: Even after intracisternal inoculation with $<10^7$ cfu of $C. \text{albicans}$, CSF cultures are only intermittently positive [31, 32]. Treatment with AmB deoxycholate cleared the CSF within a median of 1 day [32].

Our animal model is principally suitable for inference to patients: the pharmacokinetic parameters of all 4 AmB formulations in the rabbit are similar to those in humans [11, 13]. No differences are known between the 2 species regarding the susceptibility to infection by $C. \text{albicans}$, the susceptibility to brain infection, and the structural and functional properties of the blood-brain barrier.

Uptake of an antimicrobial drug into the CNS of a normal host occurs by diffusion or pinocytosis from capillaries. It is dependent on the mode of administration, blood flow, concentration of the drug, a variety of its physicochemical properties (e.g., molecular weight, size, ionization, lipid solubility, affinity to its carrier, amount of free drug, and uptake by endothelial, neuronal, and glial cells) [28, 36]. Apart from frank tissue necrosis, infection-associated inflammation damages the integrity of the tight junctions of endothelial cells at the blood-brain barrier. This disruption of the blood-brain barrier may result in increased movement of antimicrobial drugs into the CNS [36]. Indeed, hematogenous candidiasis of the brain is ultrastructurally characterized by perivascular acute inflammatory reaction and hyphal invasion of brain parenchyma and meninges [31, 37]. Tissue necrosis and pathogen-mediated perivascular inflammation may explain the higher tissue concentra-

### Table 3. Correlation between fungus burden in brain tissue and amphotericin B (AmB) concentrations in plasma and brain tissue in infected rabbits.

<table>
<thead>
<tr>
<th>Parameter of AmB concentration</th>
<th>Correlation with concentrations of Candida albicans in brain tissue ($r^2$)</th>
<th>95% confidence interval</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$</td>
<td>-0.792</td>
<td>-0.916 to -0.527</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$C_{\text{30min}}$</td>
<td>-0.815</td>
<td>-0.926 to -0.574</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AUC$_{0-24}$</td>
<td>-0.766</td>
<td>-0.905 to -0.478</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$C_{\text{mean}}$</td>
<td>-0.486</td>
<td>-0.764 to -0.055</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

$^a$ Spearman’s rank correlation.

with conventional AmB deoxycholate [13, 15]. Depending on the composition of the lipid moiety, electrical charge, particle size, and configuration, however, each of the 4 approved AmB formulations possesses unique physicochemical properties that result in distinct pharmacokinetic characteristics after iv administration. Compared with AmB deoxycholate, both AmB colloidal dispersion and AmB lipid complex, which possess complex quaternary structures, have lower $C_{\text{max}}$ levels, a shorter circulation half life in plasma, a smaller AUC, and larger volume of distribution ($V_d$), consistent with rapid and efficient uptake into tissues. In contrast, liposomal AmB, a small unilamellar liposomal formulation, is more slowly cleared from the bloodstream, has a smaller $V_d$ but achieves much higher $C_{\text{max}}$ and AUC values [11, 12, 14].

Albeit compelling, little is known about the impact of these pharmacokinetic differences on the activity of the different formulations against specific types of fungal infections and whether targeting tissue sites or escalating dosages can be used to optimize therapy with AmB. The investigation of such key relationships between pharmacokinetics and drug effects, however, is only possible in well designed animal models, which in contrast to the setting of a clinical trial, can provide a high degree of control of covariates and true outcome measurement.

In the overwhelming majority of infection models involving the 3 novel AmB formulations, dose selection has been guided by maximum tolerated dosages; AmB deoxycholate served as comparator. Only 2 studies have undertaken a head-to-head comparison of AmB deoxycholate, AmB colloidal dispersion, AmB lipid complex, and liposomal AmB for treatment of a systemic fungal infection [26, 27]. These studies uniformly described considerable differences in antifungal efficacy, depending on the formulation, the selected dosage [26, 27], and the site of infection [27].

The results of our study, which investigated the CNS disposition and pharmacodynamics of the 4 currently available AmB formulations for the first time, show a positive association of both $C_{\text{max}}$ and AUC$_{0-24}$ with the disposition of AmB in brain tissue and demonstrate that $C_{\text{max}}$, AUC$_{0-24}$, and $T_{\text{max}}$ MIC directly correlate with antifungal efficacy. These data provide a pharmacodynamic rationale for the antifungal activity of AmB deoxycholate and liposomal AmB in the treatment of fungal infections of the CNS. The data also suggest that pharmacodynamic parameters utilizing the MIC of free AmB prepared according to recommendations of the NCCLS [22], and not those of the individual formulations, correlate with antifungal efficacy at this site.
tions in infected versus noninfected rabbits receiving AmB colloidal dispersion or AmB lipid complex: In each of the 2 cohorts, 3 of 4 rabbits had ongoing brain infection at the time of autopsy. No such differences in brain-tissue levels were noted in the AmB deoxycholate– and liposomal AmB–treated rabbits: all had cleared the infection at the termination of the experiment.

Across all species, conventional AmB deoxycholate is notorious for its inability to achieve consistently measurable concentrations in CSF, both in the presence of normal and inflamed meninges [11, 32, 38, 39]. In our study, irrespective of the infection status, administration of AmB colloidal dispersion, AmB lipid complex, or liposomal AmB at 5 times higher dosages than AmB deoxycholate did not result in enhanced CSF penetration. This is consistent with the findings of other experimental [39] and clinical [40, 41] investigations. Despite mostly undetectable drug levels in the CSF, however, AmB in either formulation has activity in the treatment of experimental mycoses of the CNS [27, 42–46]. This can be explained by the achievement of therapeutically effective drug levels in brain tissue, which have been documented in laboratory animals for AmB deoxycholate [32, 39, 47], the multilamellar liposomal preparation [38], liposomal AmB [29, 48], AmB colloidal dispersion [39, 47], and AmB lipid complex [42].

The CNS remains a pharmacologic barrier to AmB regardless of formulation. From a toxicologic standpoint, this may be beneficial, since AmB therapy can be associated with significant neurotoxicity [49–53]. Although the exact mechanism of drug penetration of AmB into the CNS remain unclear, the carriers themselves do not appear to lead to an enhanced transfer of the parent compound to endothelial cells and, because of their size, they are unlikely to pass the intact blood-brain barrier [54, 55].

Our data support the idea that concentration gradients are the major determinants for drug delivery to the CNS. The 2 formulations that achieved plasma levels of AmB >2.5 above the MIC of the free compound and that maintained plasma levels above that MIC for ≥24 h (table 4) were 100% effective in eradicating C. albicans from brain tissue. Thus, those formulations that are not rapidly cleared from the bloodstream and that achieve sufficiently high levels for a sufficiently long duration may enhance delivery of free AmB to the brain either by acting as circulating depot or by entrapment in small capillaries. Additional activity may result from the leakage of vehicle-bound drug when the endothelium is damaged by infection and inflammation.

The pharmacokinetic and pharmacodynamic data of this study are consistent with the results of recent clinical trials that suggested improved activity of higher dosages (i.e., 0.7–1.0 mg/kg/day) of AmB deoxycholate and of liposomal AmB (3–6 mg/kg/day) [41, 56–59] for induction therapy of cryptococcal meningitis. The findings may also have important clinical implications for management of Candida meningoencephalitis, which historically has been difficult to eradicate by conventional antifungal therapy.

In summary, the results of this study indicate important differences among the 4 approved AmB formulations regarding drug delivery to the CNS and antifungal efficacy against ce-
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