Plasma Protein Binding of Amphotericin B and Pharmacokinetics of Bound versus Unbound Amphotericin B after Administration of Intravenous Liposomal Amphotericin B (AmBisome) and Amphotericin B Deoxycholate

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Unilamellar liposomal amphotericin B (AmBisome) (liposomal AMB) reduces the toxicity of this antifungal drug. The unique composition of liposomal AMB stabilizes the liposomes, producing higher sustained drug levels in plasma and reducing renal and hepatic excretion. When liposomes release their drug payload, unbound, protein-bound, and liposomal drug pools may exist simultaneously in the body. To determine the amounts of drug in these pools, we developed a procedure to measure unbound AMB in human plasma by ultrafiltration and then used it to characterize AMB binding in vitro and to assess the pharmacokinetics of nonliposomal pools of AMB in a phase IV study of liposomal AMB and AMB deoxycholate in healthy subjects. We confirmed that AMB is highly bound (>95%) in human plasma and showed that both human serum albumin and α1-acid glycoprotein contribute to this binding. AMB binding exhibited an unusual concentration dependence in plasma: the percentage of bound drug increased as the AMB concentration increased. This was attributed to the low solubility of AMB in plasma, which limits the unbound drug concentration to <1 µg/ml.

Subjects given 2 mg of liposomal AMB/kg of body weight had lower exposures (as measured by the maximum concentration of drug in serum and the area under the concentration-time curve) to both unbound and nonliposomal drug than those receiving 0.6 mg of AMB deoxycholate/kg. Most of the AMB in plasma remained liposome associated (97% at 4 h, 55% at 168 h) after liposomal AMB administration, so that unbound drug concentrations remained at <25 ng/ml in all liposomal AMB-treated subjects. Although liposomal AMB markedly reduces the total urinary and fecal recoveries of AMB, urinary and fecal clearances based on unbound AMB were similar (94 to 121 ml h⁻¹ kg⁻¹) for both formulations. Unbound drug urinary clearances were equal to the glomerular filtration rate, and tubular transit rates were <16% of the urinary excretion rate, suggesting that net filtration of unbound drug, with little secretion or reabsorption, is the mechanism of renal clearance for both conventional and liposomal AMB in humans. Unbound drug fecal clearances were also similar for the two formulations. Thus, liposomal AMB increases total AMB concentrations while decreasing unbound AMB concentrations in plasma as a result of sequestration of the drug in long-circulating liposomes.

Recent advances in drug delivery technology have resulted in the development of lipid-based formulations of amphotericin B, an effective but toxic fungicidal drug used in the treatment of invasive fungal infections (4). Liposomal amphotericin B (AmBisome), a liposomal formulation of amphotericin B in small, unilamellar vesicles, is significantly less toxic than the conventional amphotericin B deoxycholate formulation (5, 11, 26) and is currently indicated for the treatment of disseminated fungal infections and visceral leishmaniasis as empirical therapy for persistent febrile neutropenia. The unique therapeutic properties of liposomal amphotericin B have been linked to its ability to alter the pharmacokinetics, distribution, and excretion profiles of amphotericin B in the body (4, 8). Liposomal amphotericin B produces higher levels of drug in plasma and tissue (4–6) while markedly reducing the excretion of unchanged drug in the urine and feces (8). Although long-circulating liposomes like liposomal amphotericin B can sequester drugs in the plasma and tissue compartments, they also release drug (7) so that unbound, protein-bound, and liposomal drug pools may exist simultaneously within the body after administration. Since these pools differ in their pharmacokinetic, safety, and efficacy profiles, the measurement of total drug levels alone is not sufficient to fully characterize the bio-pharmaceutic properties of liposomal drugs (14). Amphotericin B is known to bind plasma lipoproteins (12, 27) and was recently reported to interact with serum albumin (16). However, the binding of amphotericin B to other plasma proteins and the concentration dependence of its protein binding have not been characterized. As part of a phase IV pharmacokinetic comparison of liposomal amphotericin B and amphotericin B deoxycholate (7, 8), we measured the unbound drug concentrations of amphotericin B in plasma samples from healthy volunteers after an intravenous infusion of liposomal amphotericin B. The binding of amphotericin B to human plasma and human plasma protein solutions was determined in vitro over a range of concentrations. These data were used to estimate...
and compare concentrations of amphotericin B in bound and unbound pools after administration of liposomal amphotericin B and amphotericin B deoxycholate and to determine the pharmacokinetic parameters of unbound amphotericin B for the two formulations.

MATERIALS AND METHODS

Subjects and protocol. This study was performed as part of a single-dose, open-label, randomized, parallel pharmacokinetic comparison of liposomal amphotericin B and amphotericin B deoxycholate. The protocol was reviewed and approved by an Institutional Review Board at Harris Laboratories, Lincoln, Nebr., where the study was conducted. Healthy volunteers provided informed consent prior to the study. A total of 4 males and 1 female per treatment arm completed the study. Liposomal amphotericin B-treated subjects received [14C]cholesterol liposomal amphotericin B (Gilead Sciences, San Dimas, Calif.) as a single 2-h infusion (2 mg of amphotericin B of body weight, 1 µCi of [14C]g). Amphotericin B deoxycholate-treated subjects received amphotericin B (Fungizone; Apothecon, Princeton, N.J.) as a single 2-h infusion (0.6 mg of amphotericin B/kg). Amphotericin B deoxycholate-treated subjects also received an intravenous test dose of amphotericin B (1 mg) 1 day prior to the study dose. All subjects were premedicated with acetaminophen (650 mg) and diphenhydramine (25 mg). Subjects were maintained on study medications (Fungizone) throughout the study.

Sampling of plasma, urine, and feces. Plasma was obtained at 0.5, 1, 2, 2.5, 3, 4, 6, 8, 10, 18, 24, 36, 60, 94, 120, 144, and 168 h after the start of the 2-h infusion. Urine and feces were continuously collected during the 1-week study. Samples were analyzed for total amphotericin B by high-performance liquid chromatography and liquid chromatography tandem mass spectrometry (LC/MS/MS) (2, 8). Aliquots of freshly separated EDTA plasma obtained from liposomal amphotericin B-treated subjects at the 2.25-h, 4-, 10-, 24-, 60-, 96-, 120-, 144-, and 168-h time points were placed into centrifugal micropartition devices which had a molecular mass cutoff of approximately 30 kDa (Centrifree; Amicon, Beverly, Mass.). The devices were centrifuged for 30 min at 2,500 rpm. The resulting plasma ultrafilters were collected and frozen at −20°C until analyzed for amphotericin B.

Preliminary investigation of ultrafiltration and equilibrium dialysis for determination of unbound amphotericin B. Prior to the main study, the abilities of two methods, ultrafiltration and equilibrium dialysis, to determine the unbound drug concentrations of amphotericin B were investigated. Pooled human EDTA plasma spiked with amphotericin B (ICN Biomedicals, Costa Mesa, Calif.) in dimethyl sulfoxide to final concentrations of 0.1, 1.5, 15, 30, and 75 µg/ml (the final dimethyl sulfoxide concentration was <0.8%) and incubated for 1 h at 37°C. Unbound amphotericin B concentrations were determined in ultrafiltrates and dialysates obtained from each sample. For ultrafiltration, spiked plasma was centrifuged in a micropartition device with a 30-kDa cutoff (Centrifree) for 30 min at 2,500 rpm. Equilibrium dialysis was performed in a 1-ml Tefflon cell with a 1,500-Da cutoff (Spectra/Permeate; Spectrum Laboratories, Rancho Dominguez, California) against Krebs-Ringer buffer for 8 h at 37°C. The total amphotericin B concentrations in spiked plasma (C_{TOT}), ultrafiltrate (C_{UF}), and dialysate (C_{UF}) were determined by LC/MS/MS as described below. For ultrafiltration, the percentage of bound drug was determined using the following formula: % bound = 100 × (C_{TOT} − C_{UF})/C_{TOT}. For equilibrium dialysis, the percentage of bound drug was determined according to the following formula: % bound = 100 × (C_{TOT} − C_{UF})/C_{TOT}. These formulas were used to correct for dilution of the dialysate by buffer. Because this preliminary study showed that the measured binding of amphotericin B was similar for both methods, subsequent portions of the study employed only ultrafiltration to determine unbound drug concentrations of amphotericin B.

Concentration dependence of amphotericin B plasma binding. To determine the concentration dependence of amphotericin B plasma binding in plasma, amphotericin B-spiked human samples (0.1, 1.5, 15, 30, and 75 µg of amphotericin B/ml) was prepared in human EDTA plasma as described above. Quadruplicate samples of each concentration were subjected to ultrafiltration as described above. Ultrafilters and unfiltered samples were analyzed by LC/MS/MS to determine the unbound and total concentrations of amphotericin B and the percentage of bound drug in plasma at each concentration.

Amphotericin B binding to human plasma protein solutions. Amphotericin B is highly bound to plasma lipoproteins (12). To determine the contribution of other proteins to amphotericin B binding in human plasma, solutions containing 4% human serum albumin (HSA) (99% purity; Sigma Chemical Co.) and 0.1% human α₁-acid glycoprotein (AAG) (99% purity; Sigma Chemical Co.) were prepared in Krebs-Ringer buffer. Quadruplicate samples of each protein solution were spiked with 5 and 30 µg of amphotericin B/ml subjected to ultrafiltration as described above, and analyzed by LC/MS/MS for amphotericin B to determine the percentage of bound drug.

Amphotericin B assays. Total amphotericin B concentrations in spiked plasma and plasma protein samples from the in vitro binding studies were determined by LC/MS/MS (20). Amphotericin B concentrations in ultrafilters and dialysates were measured by a modification of this method. Ultrafiltrate and dialysate samples (0.2 ml) were deproteinized with methanol (0.4 ml) and diluted in water (1 ml), and injected onto a solid-phase extraction column (Isolute C18 EC; Jones Chromatography, Lakewood, Colo.). Amphotericin B was eluted with methanol, reconstituted in methanol, and diluted with internal standard (natamycin; USP). Standards were separated on a reverse-phase column (3-µm particle size, 3-mm internal diameter, 150 mm long; Symmetry C18, Waters Corp., Milford, Mass.) by using a mobile phase of methanol-water-acetic acid (69:29:2) and detected by using a Sciex API 3000 tandem mass spectrometer in positive-ion mode. For analysis of the subjects’ plasma ultrafiltrate samples, standards (1 to 200 ng/ml) were prepared in blank plasma. For analysis of in vitro study ultrafilters and dialysates, standards (0.01 to 10 µg/ml) were prepared in blank ultrafiltrate or Krebs-Ringer. The assay had a validated range of 1.0 to 200 ng/ml. Samples were diluted into this range as required. Interday and intraday accuracies were within ±14%, while interday and intraday precisions were ≤16% (coefficient of variation) over the range of the assay.

Estimation of amphotericin B in various plasma pools. When unbound amphotericin B was measured in ultrafilters of spiked human plasma, the percent bound drug was determined using nonlinear function curve estimation. A linear regression fit to these data was used to estimate the concentration of unbound amphotericin B in each liposomal amphotericin B-treated subject’s plasma from the measured unbound amphotericin B concentrations. The concentration of nonliposomal amphotericin B (C_{unlip}) and the percentage of bound drug concentration (C_{TOT}) was fit to an equation of the form C_{TOT} = (A × C_{unlip})/(B + C_{unlip}) using a nonlinear, least-squares parameter estimation method (PSI-Plot; Poly Software, Salt Lake City, Utah). The calculated parameters, A and B, were used to estimate concentrations of unbound amphotericin B (C_{unbound}) in plasma from amphotericin B deoxycholate-treated subjects based on the total amphotericin B concentrations measured in their plasma (C_{TOT}).

Pharmacokinetic and statistical analysis. Pharmacokinetic parameters based on total amphotericin B concentrations in plasma and on urinary and fecal excretion of unchanged amphotericin B were determined as previously described (8, 25). Pharmacokinetic parameters based on unbound amphotericin B concentrations in plasma were determined from the unbound drug concentrations measured in liposomal amphotericin B-treated subjects and calculated in amphotericin B deoxycholate-treated subjects. Clearances of unbound amphotericin B (CL_{unbound}) were calculated as CL_{unbound} = dose/AUC_{unbound}, where AUC_{unbound} is the area under the curve of unbound amphotericin B in plasma, determined by linear trapezoidal integration to the last time point. Urinary and fecal clearances of unbound amphotericin B (CL_{urine} and CL_{feces}, respectively) were similarly calculated as CL_{urine} = A_{urine}/AUC_{urine} and CL_{feces} = A_{feces}/AUC_{urine}, where A_{urine} and A_{feces} were the areas under the respective unchanged in the urine and feces, respectively. The glomerular filtration rate (GFR) for each subject was estimated from the subject’s body surface area (24). The tubular transit rate, T, a quantitative measure of the net tendency for renal secretion or reabsorption (3), was calculated as T = UER − (W_{f} × C_{TOT} × GFR), where UER was the urinary excretion rate (UER = A_{urine}/168 h), and W_{f} was the fraction of water in plasma (W_{f} = [100 − total protein]/100). A positive value for T indicates net secretion, a negative value indicates net reabsorption, and a value of zero indicates no net secretion or reabsorption (3).

Liver plasma flow (LPF) was estimated for each subject from published values (13). The statistical significance of differences in parameters between treatments was evaluated using the unpaired two-sample t test with a significance level of 0.05 (two tailed). The t test for unequal variances was applied in cases where the F test for variances showed group variances to be unequal. Due to the small number of females in the study, the data were analyzed without regard to gender.
TABLE 1. Amphotericin B binding in vitro to human plasma and plasma protein solutions determined by ultrafiltration and equilibrium dialysis

<table>
<thead>
<tr>
<th>Matrix</th>
<th>AMB concn in matrix (μg/ml)</th>
<th>% of bound drug determined by:</th>
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<tr>
<td></td>
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<td>Ultrafiltration</td>
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<tr>
<td>Plasma</td>
<td>0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>1.34</td>
<td>96.3</td>
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<td>99.0</td>
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<tr>
<td></td>
<td>74.3</td>
<td>99.5</td>
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<tr>
<td>HSA (4%)</td>
<td>2.44</td>
<td>95.5</td>
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<td></td>
<td>14.8</td>
<td>93.4</td>
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<tr>
<td>AAG (0.1%)</td>
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<td>90.0</td>
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<td></td>
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AMB, amphotericin B; ND, not determined. Values are means of all samples tested.

RESULTS

This study used ultrafiltration to characterize the in vitro binding of amphotericin B to human plasma and plasma proteins and to measure unbound amphotericin B in the plasma of subjects who received liposomal amphotericin B. From these data, the pharmacokinetics of unbound amphotericin B were determined as part of a phase IV study of liposomal amphotericin B and amphotericin B deoxycholate pharmacokinetics in healthy human volunteers.

In vitro binding studies. A preliminary in vitro study compared two methods, ultrafiltration and equilibrium dialysis, for measuring unbound amphotericin B concentrations in spiked human plasma. Both methods gave similar estimates for the extent of amphotericin B protein binding (Table 1). Based on these findings, ultrafiltration was used to measure unbound amphotericin B concentrations in the remaining portions of the in vitro and phase IV studies.

In the next phase of the in vitro study, human plasma was spiked with amphotericin B over a range of concentrations, and the percentage of bound drug was determined from measurements of total and ultrafiltrate concentrations (Fig. 1, upper panel). Amphotericin B binding increased from 95.3% at its lowest total concentration (0.62 μg/ml) to 99.2% at its highest concentration (65 μg/ml). The percentage of bound drug appeared to increase linearly with the unbound drug concentration over the range of concentrations studied (Fig. 1, lower panel). A linear regression fit to the data gave a y-axis intercept of 95.5% bound (at a concentration of zero) and a slope of 0.00606. The fit also predicted that the maximum concentration of unbound amphotericin B (as binding approaches 100%) would be 744 ng/ml.

In the last phase of the in vitro study, the binding of amphotericin B to solutions of HSA and AAG, at approximately physiologic concentrations of these proteins, was determined by ultrafiltration (Table 1). Amphotericin B bound strongly (>90%) to HSA and AAG, indicating that both proteins could act as carriers of amphotericin B in human plasma.

Plasma profiles of unbound and nonliposomal amphotericin B pools. Concentrations of unbound amphotericin B in the plasma of liposomal amphotericin B-treated subjects were determined by measuring amphotericin B in ultrafiltrates of plasma at time points between 0.25 and 168 h after adminis-

FIG. 1. Binding of amphotericin B (AMB) in human plasma measured by ultrafiltration. The upper panel shows the percentage of bound drug versus the total concentration (C_{TOT}), with the line showing a nonlinear fit to the data (% bound = 100 - [A/(B + C_{TOT})]). The lower panel shows the percentage of bound drug versus the unbound (ultrafiltrate) concentration, with the line showing a linear regression fit to the data.

FIG. 2. Amphotericin B (AMB) concentrations (means ± standard deviations) in various pools in plasma after a 2-h infusion of 2 mg of liposomal amphotericin B/kg in healthy subjects.
tation (Fig. 2). Unbound amphotericin B concentrations did not exceed 25 ng/ml at any time point in any subject given liposomal amphotericin B. Mean unbound drug concentrations fell from 14.5 ± 5 ng/ml at 15 min after the end of the infusion, to below 5 ng/ml by 24 h, and they remained between 2.5 and 5 ng/ml for the rest of the 1-week study. Unbound amphoter-

Cmax (μg/ml)        0.016 ± 0.004  0.060 ± 0.01  <0.01
Tmax (h)            3.5 ± 0.96   2.0 ± 0.007  <0.05
AUCunbound (μg · h ml⁻¹)  0.74 ± 0.13  1.22 ± 0.13  <0.01
CLunbound (ml h⁻¹ kg⁻¹)  121.9 ± 14.2  94.2 ± 15.3  <0.05
CLp/GFR ratio    1.10 ± 0.08  0.84 ± 0.10  <0.01
UER (μg h⁻¹)     40.0 ± 7.08  87.3 ± 11.3  <0.01
T (μg h⁻¹)       6.0 ± 2.53   −7.3 ± 1.16  NS
CLu/LPF ratio   0.16 ± 0.15  0.20 ± 0.11 NS

For Liposomal
  amphotericin B (2 mg/kg)  Amphotericin B
deoxycholate (0.6 mg/kg)  p

a Tmax, time at which Cmax occurred; NS, not significant.
b Values are means ± standard deviations (n = 5).
c n = 4 (incomplete fecal collection in one subject).

The urinary recovery of unchanged amphoter-

that was liposome associated fell slowly over the course of the study, from 98 ± 1.4% at the end of the infusion to 55 ± 10% at the end of the 1-week study (Fig. 4).

Pharmacokinetic parameters of unbound amphotericin B. Unbound amphotericin B concentrations in liposomal amphotericin B-treated subjects were determined by measuring amphotericin B in plasma ultrafiltrates. As a comparison, concentrations of unbound amphotericin B in plasma in amphotericin B deoxycholate-treated subjects were calculated from measured total amphotericin B concentrations and in vitro percent binding data. An infusion of 2 mg of liposomal amphotericin B/kg resulted in lower exposures to unbound amphotericin B than did an infusion of 0.6 mg of amphotericin B deoxycholate/kg (P < 0.01 for Cmax and AUC) (Table 2). Renal and fecal clearances of the two formulations were then calculated in terms of their unbound drug concentrations (Table 2). Although the urinary recovery of unchanged amphoter-
Amphotericin B differed markedly between liposomal amphotericin B and amphotericin B deoxycholate (4.5 versus 20.6% of the dose, respectively), the urinary clearances of unbound amphotericin B were similar for the two formulations. Both formulations had unbound urinary clearances that were within 16% of the subjects’ GFR (Table 2). The tubular transit rate (T) was generally positive for liposomal amphotericin B-treated subjects and negative for amphotericin B deoxycholate-treated subjects, but these values represented only a small fraction (<15%) of the total urinary excretion rate and did not differ between treatments (P > 0.05). Thus, no indication of significant net tubular secretion or reabsorption was observed. Similarly, although fecal excretion of unchanged amphotericin B differed markedly between liposomal amphotericin B and amphotericin B deoxycholate (4.0 versus 42.5%, respectively), the fecal clearances of unbound amphotericin B did not appear to differ between the two formulations (P > 0.05). Observed fecal clearances were between 16 and 20% of the subjects’ estimated LPF.

**DISCUSSION**

This study found that liposomal amphotericin B increases total concentrations of amphotericin B in plasma while it decreases unbound amphotericin B concentrations. This study also demonstrated that amphotericin B is highly bound to human plasma and that albumin and AAG contribute to this binding. Amphotericin B binding in plasma was distinctly nonlinear and concentration dependent. Subjects receiving liposomal amphotericin B had significantly lower exposures to unbound and nonliposomal drug, as measured by the C\textsubscript{max} and AUC, than did those receiving amphotericin B deoxycholate. Among subjects receiving liposomal amphotericin B, the preponderance of amphotericin B in plasma was liposome associated, such that unbound drug concentrations remained at <25 ng/ml in all subjects during the course of the study. Liposomal amphotericin B markedly reduced total urinary and fecal recoveries of amphotericin B. However, the urinary and fecal clearances of unbound amphotericin B were similar in subjects receiving liposomal amphotericin B or amphotericin B deoxycholate. Urinary clearances of unbound amphotericin B were similar to the GFR, and tubular transit rates were relatively small, suggesting that unbound amphotericin B is excreted via filtration with minimal secretion or reabsorption.

The in vitro study of amphotericin B binding to human plasma and plasma proteins confirms and extends the results of previous studies showing that amphotericin B binds strongly to plasma and to plasma lipoproteins (9, 12, 27). In this study, amphotericin B was shown, by both ultrafiltration and equilibrium dialysis, to be >95% bound over a range of concentrations in human plasma. Amphotericin B also bound strongly to two major plasma binding proteins, HSA and AAG, with over 90% bound at physiologic concentrations of these proteins. These results are consistent with the observation that a majority of the amphotericin B in plasma is found in the lipoprotein-deficient fraction (16).

The in vitro study also demonstrated the unusual concentration dependence of amphotericin B binding in human plasma. For most drugs, the percentage of bound drug decreases with increasing plasma drug concentration as a result of binding site saturation. In contrast, the binding of amphotericin B in plasma increased with increasing drug concentration, from approximately 95% bound at 0.6 μg/ml to over 99% bound at 65 μg/ml. A previous study, using only two amphotericin B concentrations, showed similar behavior (9). The observed concentration dependence represents a nearly fivefold change in the unbound fraction of the drug over the range of concentrations studied, suggesting that the apparent clearance and volume of distribution of amphotericin B could change significantly as the unbound fraction changes with concentration.

The mechanism for the unique concentration-dependent binding of amphotericin B remains undetermined. One possible explanation for this behavior is that the concentration of unbound amphotericin B is limited by its low solubility, while the capacity of plasma proteins to bind amphotericin B is much larger. Proteins known to bind amphotericin B (lipoproteins, HSA, and AAG) together constitute >5% of the plasma mass, suggesting that binding remains unsaturated at the concentrations tested. Extrapolation of the apparently linear relationship between the percentage of bound drug in plasma and ultrafilterate concentration suggests that amphotericin B has a solubility of less than 1 μg/ml in plasma water. As the unbound amphotericin B concentration in plasma approaches this limit, the addition of further drug would increase only the amount bound to proteins, and the percentage of bound drug would increase.

Previous studies suggested that amphotericin B solutions contain both monomers and multimolecular aggregates or micelles (17, 22, 23) and that these forms may have different pharmacologic properties (21). This study did not attempt to differentiate these putative species, but it measured the fraction of total amphotericin B that could freely diffuse through semipermeable membranes. This provides an appropriate physiologic estimate of the fraction of amphotericin B in plasma that is subject to renal filtration at the glomerulus for use in the analysis of unbound drug pharmacokinetics. The fact that recovery did not differ between the 30-kDa ultrafiltration membrane and the 12- to 14-kDa dialysis membrane suggests that the unbound fraction of amphotericin B in plasma consisted mainly of low-molecular-mass species. This is consistent with the observation that unbound renal clearances of both amphotericin B formulations were essentially equal to the GFR.

Unbound amphotericin B concentrations in liposomal amphotericin B-treated subjects were compared to unbound drug concentrations in amphotericin B deoxycholate-treated subjects. Likewise, nonliposomal amphotericin B concentrations in liposomal amphotericin B-treated subjects were compared to total amphotericin B concentrations in amphotericin B deoxycholate-treated subjects. In both cases, liposomal amphotericin B produced lower exposures to the unencapsulated pools of drug, while producing higher exposures to total amphotericin B. This behavior is consistent with the observation that amphotericin B in the plasma remains largely liposome associated even 1 week after administration (7). Together, these data support the hypothesis that liposomal amphotericin B is a stable, long-circulating liposomal delivery system that sequesters its amphotericin B payload in the plasma, slowly releasing it over time as well as targeting the drug to tissues and fungal targets via the uptake of intact liposomes (1, 7, 8).
For at least 1 week after liposomal amphotericin B administration, levels of liposomal drug in plasma were higher than those of nonliposomal drug, demonstrating the ability of this formulation to provide a prolonged reservoir of encapsulated drug in the plasma compartment.

The reduced exposures to unbound amphotericin B in liposomal amphotericin B-treated subjects are consistent with the observed reductions in renal and fecal excretion of unchanged drug, reduced drug distribution to the kidneys (1, 6, 19), and reduced nephrotoxicity (25, 26) of liposomal amphotericin B, suggesting that all these processes depend upon the concentration of diffusible or unbound drug in the plasma. In this study, renal and fecal clearances based on unbound drug concentrations of amphotericin B in the plasma were nearly identical for both liposomal amphotericin B and amphotericin B deoxycholate. Thus, the lower combined renal and fecal excretion of liposomal amphotericin B (8.5%) versus amphotericin B deoxycholate (63%) may result from the lower exposures to unbound amphotericin B after liposomal amphotericin B treatment. While liposomal amphotericin B increases total concentrations of amphotericin B in plasma, most of this represents drug encapsulated in the long-circulating liposomal pool, where it is protected from renal filtration and fecal elimination but may still be accessible to fungal targets (1).

The analysis of unbound amphotericin B pharmacokinetics in this study also provided new information on the mechanism of amphotericin B renal elimination. Renal clearances based on unbound amphotericin B concentrations were nearly equal to the GFR for both amphotericin B formulations. Moreover, the tubular transit rate, a quantitative measure of net renal filtration or reabsorption, was only a fraction of the total urinary excretion rate for either formulation. Thus, glomerular filtration of the unbound drug fraction in plasma followed by excretion of unchanged drug can account for a majority of the renal elimination of amphotericin B. Renal secretion, reabsorption, and metabolism appear to play a small role, if any, in the elimination of amphotericin B in humans. This analysis suggests that the apparent renal clearances of liposomal amphotericin B and amphotericin B deoxycholate could be sensitive to changes in either GFR or protein binding.

Although the renal clearance of amphotericin B appears to be largely passive, fecal excretion rates indicate that the drug is actively secreted into the bile (18). Fecal clearances of unbound amphotericin B were approximately 20% of LPF for both formulations, indicating that the liver extracts only a portion of the unbound amphotericin B in plasma. Because of this moderate extraction ratio, the hepatic clearance of amphotericin B is not likely to be sensitive to changes in liver blood flow.

The similarity of unbound amphotericin B clearances between liposomal amphotericin B and amphotericin B deoxycholate also suggests that renal and fecal clearance occurs after the release of amphotericin B from the liposomes in liposomal amphotericin B-treated subjects. If liposomal amphotericin B is cleared via its unbound drug pool, then excretion of intact liposomes does not occur by these routes and liposomal drug taken up into tissues may be eliminated by another mechanism, such as metabolism, or be released very slowly from the tissues. The long tissue residence times of liposomal drugs (15) and failure to detect amphotericin B metabolites (8) are consistent with the latter hypothesis.

Previous studies of liposomal amphotericin B pharmacokinetics have been based on total drug concentrations in plasma (6, 10, 25). In this study, the plasma profiles of total, liposomal, nonliposomal, and unbound drug pools were individually characterized. Because the concentrations of nonliposomal drug were small compared to total amphotericin B concentrations, the plasma profiles of total and liposomal drug were similar in liposomal amphotericin B-treated subjects (Fig. 2). Thus, previous estimates of liposomal amphotericin B pharmacokinetic parameters should closely approximate the pharmacokinetics of the liposomal drug pool, especially during daily dosing regimens. The nonliposomal and unbound drug pools had much lower concentrations but longer terminal half-lives than the liposomal pool after liposomal amphotericin B administration, consistent with the slow washout of amphotericin B from tissue reservoirs.

In summary, the in vitro study demonstrated that amphotericin B is highly bound (>95%) over a range of concentrations in human plasma, with both HSA and AAG contributing to this binding. Due to its limited solubility, amphotericin B binding exhibits a unique concentration dependence; the percentage of bound drug in plasma increases with amphotericin B concentration. In the phase IV study, 2 mg of liposomal amphotericin B/kg produced lower exposures to unbound and nonliposomal drug than did 0.6 mg of amphotericin B deoxycholate/kg, with a majority of the amphotericin B remaining unliposomal 1 week after liposomal amphotericin B administration. While the excretion profiles of liposomal amphotericin B and amphotericin B deoxycholate differ markedly, their unbound renal and fecal clearances were similar, demonstrating that renal and fecal clearance occurs via unbound drug for both formulations. Liposomal amphotericin B increases total drug concentrations, while decreasing unbound drug concentrations in plasma as a result of sequestration of the drug in long-circulating liposomes.

REFERENCES


