Behavior of Amphotericin B Lipid Complex in Plasma In Vitro and in the Circulation of Rats

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Amphotericin B lipid complex (ABLC) shows reduced toxicity relative to that of amphotericin B deoxycholate (AmB-d) while maintaining antifungal activity. Rat blood or plasma was spiked with ABLC in vitro. Released amphotericin B was separated from the parent material by centrifugation. At early times (0 to 15 min) most (~90%) of the amphotericin B was complexed. The amount of released amphotericin B increased gradually in a time- and temperature-dependent fashion. The released amphotericin B was associated with plasma lipoprotein and nonlipoprotein proteins. The area under the concentration-time curve from 0 to 24 h for total amphotericin B in whole blood of rats given a single intravenous bolus dose of 1 mg of ABLC per kg of body weight was fourfold lower than that in rats given 1 mg of AmB-d per kg. The complexed amphotericin B was rapidly removed from the circulation and was distributed to the tissues in these rats. Other rats were treated intravenously with ABLC (10 mg/kg/day) or AmB-d (0.5 mg/kg/day) daily for 15 days. Blood was collected at 15 and 180 min after administration of the last dose. The total levels of amphotericin B in the blood of the group given ABLC were about three to five times those in the group given AmB-d, and the concentration of released, protein-bound amphotericin B in the plasma of the group given ABLC was about one to two times that observed for the group given AmB-d, despite the 20-fold difference in dose. The relative protein distribution of amphotericin B in plasma was similar after ABLC or AmB-d administration under these steady-state conditions in vivo. The rapid uptake of complexed amphotericin B by tissues and the very low levels of circulating protein-bound amphotericin B in plasma after the administration of ABLC may explain, in part, the reduced toxicity and enhanced therapeutic index of this preparation.

Amphotericin B has been the agent of choice for the treatment of serious fungal infections for more than 30 years. However, administration of the most common preparation of amphotericin B (AmB-d; a sodium deoxycholate colloidal suspension [Fungizone]) is associated with severe, dose-limiting, acute, and chronic toxicities, particularly nephrotoxicity. Studies with animals and humans have indicated that a lipid-complexed preparation of amphotericin B is less toxic than AmB-d and has an enhanced therapeutic index (6). Amphotericin B lipid complex (ABLC) is a suspension of amphotericin B complexed with the lipids 1,2-dimyristoylphosphatidylglycerol (DMPG) and 1,2-dimyristoylphosphatidylglycerol (DMPG) with a mean particle diameter of approximately 3 μm. ABLC appears as ribbons by freeze-fracture electron microscopy (12), and a model for its supramolecular structure has been proposed (13). In single-dose, acute toxicology studies in rodents, ABLC has been shown to be 10- to 20-fold less toxic than AmB-d (6). In repeated-dose studies in beagle dogs, nephrotoxicity (judged by urea nitrogen and creatinine levels in serum as well as renal histology) was 8- to 10-fold less than that seen with AmB-d (13).

ABLC has been studied against a variety of experimental fungal infections (6-8, 15, 18, 20), and in no case was ABLC shown to be less effective than AmB-d. For those infections against which AmB-d is effective, the dose of ABLC (in terms of amphotericin B content) necessary to produce comparable results was usually found to be similar or slightly greater. However, since the toxicity of ABLC is generally 10-fold less than that of conventional amphotericin B, there is a much greater window of therapeutic efficacy with the use of ABLC in the treatment of mycotic infections. For instance, in some models in which efficacy could not be achieved with the maximum tolerated intravenous dose of AmB-d, it was possible to achieve efficacy with ABLC since much higher doses could be given safely. In clinical trials, ABLC has been shown to be at least as effective as AmB-d but significantly less nephrotoxic (1). Additionally, ABLC has been shown to be effective in patients who had failed to respond to AmB-d (22).

At comparable amphotericin B doses, the concentrations in tissue following ABLC administration are reported to be much higher than those obtained after AmB-d administration, while the concentrations in serum or blood are lower (10, 14, 17). This difference in the pharmacokinetics and distribution of ABLC and AmB-d could be explained if the amphotericin B-lipid complex remained intact in the circulation. The particular nature of ABLC would then result in rapid uptake by tissues, especially those in areas where the normal capillary endothelium is disrupted (by fungal invasion or inflammation, for example) or by the reticuloendothelial system. Release of active (monomeric) amphotericin B would then occur within selected tissues as a result of fungal or host-derived phospholipases (13, 21) or phagocytic digestion. Thus, the enhanced therapeutic index of ABLC might be due to the altered pharmacokinetics and targeted release of amphotericin B with this formulation. Other explanations, however, are possible. It has been proposed that the enhanced therapeutic index of certain liposomal preparations of amphotericin B is due to an alteration in the distribution of the amphotericin B among the different serum lipoprotein classes (2, 24). After intravenous injection of AmB-d, the amphotericin B in plasma is extensively protein bound (>90%), is nondialyzable, and binds to
lipoproteins as well as other plasma proteins (3–5). Amphotericin B by itself or as AmB-d incubated with human serum in vitro was shown to bind preferentially to very low density lipoprotein (VLDL) and low-density lipoprotein (LDL), whereas the amphotericin B from liposomal amphotericin B bound preferentially to high-density lipoprotein (HDL). The amphotericin B associated with HDL is reported to be less toxic to renal cells in vitro than the amphotericin B associated with LDL (23).

This study was designed to determine the behavior of amphotericin B when administered as ABLC in the circulation of rats and to compare this with the behavior of amphotericin B administered as AmB-d. Initial in vitro studies were conducted to determine if ABLC remains intact or if amphotericin B separates from the complex in plasma and to assess the fate of released or noncomplexed amphotericin B. Subsequent studies involved collection of blood from rats given single or multiple doses of ABLC. In all studies, the complexed amphotericin B was separated from the released amphotericin B by centrifugation. In some studies, differential ultracentrifugation was used to separate lipoprotein classes prior to quantitation of amphotericin B by high-pressure liquid chromatography (HPLC).

(Materials and Methods)

Materials. ABLC (ABELCET) was obtained from The Liposome Co., Inc., Princeton, N.J. AmB-d (Fungizone) was purchased from Bell Medical (Edison, N.J.). The doses of both ABLC and AmB-d are given in terms of amphotericin B content. N-Acetylamphotericin B was synthesized by a modification of previously described methods (19). Potassium bromide, sodium chloride, and EDTA (sodium salt) were obtained from Sigma Chemical Co. (St. Louis, Mo.). Methanol, acetonitrile, chloroform, dimethyl sulfoxide, and HPLC-grade water were obtained from J. T. Baker Inc. (Phillipburg, N.J.). The total cholesterol and the biuretichronic acid (BCA) protein assay kits were obtained from Sigma Chemical Co. and Pierce, respectively.

Separation of complexed amphotericin B from released amphotericin B in ABLC-spiked samples in vitro. Fresh EDTA-anticoagulated blood was collected from Sprague-Dawley rats (males; weight, 350 to 400 g; Charles River Breeding Laboratories, Raleigh, N.C., and Kingston, N.Y.) by cardiac puncture (10-ml Monovettes with EDTA; Sarstedt) while the rats were under CO2 anesthesia. Plasma was obtained by centrifugation. Saline, pooled blood, or plasma was spiked with ABLC or AmB-d at amphotericin B concentrations ranging from 0.31 to 10.0 μg/ml, and the spiked samples were incubated at 25 or 37°C for 0, 30, 60, and 180 min. At the end of the time interval, samples were immediately centrifuged at 1,500 × g at ambient temperature for 10 min in a Sorvall T10 centrifuge equipped with a swinging-bucket rotor. The supernatant was removed and the pellet was resuspended in 1 ml of 0.9% sodium chloride (Abbott Laboratories). The pellets from plasma and saline and the supernatants from blood, plasma, and saline were analyzed for amphotericin B concentration by HPLC.

Identification of complexed amphotericin B. Complexed amphotericin B was identified by circular dichroism (CD) spectroscopy. The spectra were obtained with a Jasco J710 spectropolarimeter equipped with a 0.1-cm-path-length flow cell. Each spectrum represented the average of three or five scans obtained over a wavelength range of 250 to 500 nm.

Amphotericin B quantitation. Amphotericin B concentrations were determined by HPLC of sample extracts (25). Whole-blood samples (200 μl) were extracted with 600 μl of acetonitrile-chloroform-dimethyl sulfoxide (30:30:40; vol/vol) containing the internal standard, N-acetylamphotericin B at 0.5 μg/ml. Supernatants of the plasma samples (200 μl) were extracted with methanol containing 0.5 μg of the internal standard, N-acetylamphotericin B, per ml (600 μl). Chromatography was performed with a Waters 626 pump, 600S controller, 717 plus autosampler, and 996 PDA detector (Waters, Milford, Mass.). The mobile phase consisted of acetonitrile–0.0875 M EDTA (36:64; vol/vol) at a flow rate of 1.0 ml/min. Separation was performed by reverse-phase chromatography on a Bondapak C18 column (3.9 by 150 mm; 10 μm; Waters). Detection was by measurement of the absorbance at 405 nm. The standard curves for whole blood and plasma were linear from 0.050 to 5.00 μg/ml, with a correlation coefficient of 0.998 or greater. By the whole-blood assay, the relative standard deviations at 0.050 and 4.00 μg/ml were 15.45 and 2.90%, respectively, for AmB-d and 5.37 and 4.59%, respectively, for ABLC. By the plasma assay, the percent relative standard deviations at 0.050 and 4.00 μg/ml were 7.91 and 6.03%, respectively, for AmB-d.

Behavior of amphotericin B after administration of single or repeated doses of ABLC or AmB-d to rats. All animals were acclimated for at least 5 days prior to surgery and dosing. For the single-dose study, medical grade implants were surgically implanted in the abdominal aorta of 14 male Sprague-Dawley rats (weight, 320 to 350 g) under sodium pentobarbital anesthesia. The cannulas were flushed with 0.1 ml of 20 U of heparin per ml to maintain patency. Rats were allowed to recover from the surgery for 24 to 48 h before administration of a single dose of ABLC (seven rats) or AmB-d (seven rats) via the tail vein as an intravenous bolus of 1 mg/kg of body weight. The ABLC (5 mg/ml in saline) was filtered through a Monoject 305 filter needle (5 μm) and was diluted to 1 mg/ml with 5% dextrose prior to injection. AmB-d was reconstituted with sterile water to 5 mg/ml and was further diluted to 1.0 mg/ml with 5% dextrose. Serial blood samples (with EDTA used as an anticoagulant) were collected from the aortic cannulas at 5 and 15 min and 1, 3, 6, 12, and 24 h post dosing. An aliquot of 200 μl was removed from each blood sample and was frozen at −70°C for later determination of the total amphotericin B concentration by HPLC. The rest of the blood sample was immediately centrifuged to obtain plasma, which was recentrifuged to obtain plasma supernatant. The amphotericin B in the plasma supernatants was quantified by HPLC. The area under the concentration-time curve (AUC) was calculated for each rat by using the trapezoidal rule.

Two additional groups of six rats each (weight, 320 to 350 g at the start of treatment) were administered the maximum tolerated dosages of either ABLC (100 mg/kg/day) or AmB-d (0.5 mg/kg/day) via the tail vein for 15 days. On day 15, blood (approximately 10 ml) was collected from each rat by cardiac puncture into Monovettes containing EDTA at either 15 or 180 min following administration of the last dose. An aliquot of 250 μl was removed from each 10-ml blood sample and was frozen at −70°C for later determination of the amphotericin B concentration by HPLC. The rest of the blood sample was centrifuged to obtain plasma, which was recentrifuged to obtain plasma supernatant and was then immediately fractionated by ultracentrifugation to separate the lipoprotein classes. The amphotericin B concentration in each fraction was measured by HPLC.

Separation of lipoproteins and other plasma proteins. Approximately 4 ml of the plasma supernatant was fractionated to obtain the lipoproteins of different densities by a sequential method based on the density gradient ultracentrifugation technique (11). The following density ranges defined the lipoproteins: VLDL, 1.006 g/ml; LDL, 1.019 to 1.063 g/ml; and HDL, 1.063 to 1.211 g/ml. Potassium bromide and sodium chloride were used for the density adjustments. The lipoproteins were separated sequentially after each density adjustment and approximately 20 to 24 h of centrifugation (approximately 100,000 × g; Beckman model L5-50E ultracentrifuge). The proteins that did not separate at the density adjustment of 1.211 g/ml are referred to as the “other” plasma proteins. These are nonlipoprotein proteins such as albumin and various glycoproteins. The VLDL, LDL, and HDL fractions as well as the other plasma protein fractions were analyzed by HPLC for the determination of amphotericin B concentration.

Cholesterol and protein measurements. For both the in vitro and in vivo studies, fractions containing lipoproteins of different densities and other plasma proteins were analyzed for total cholesterol and protein concentrations. The cholesterol determinations were performed by a colorimetric assay based on an enzymatic reaction (Sigma), and the protein concentrations were determined spectrophotometrically by a BCA protein assay (Pierce).

RESULTS

Separation of complexed amphotericin B from released amphotericin B in plasma and blood. When warm saline was spiked with ABLC at 1.0 μg/ml, virtually all of the amphotericin B was pelleted by low-force centrifugation, as evidenced by the lack of amphotericin B in the supernatant at all time points studied (Fig. 1). When ABLC at the same concentration was added to fresh rat plasma or whole blood, amphotericin B appeared in the supernatant in a time- and temperature-dependent fashion. Immediately after spiking the plasma, only 1.3% of the amphotericin B was found in the supernatant, whereas after 3 h at 37°C, 73% of the amphotericin B was found in the supernatant. The recovery of amphotericin B in the pellet or supernatant of plasma samples spiked with ABLC was concentration dependent (Table 1).

The behavior of ABLC in plasma in vitro was compared to that of AmB-d (Table 2). ABLC or AmB-d was added to fresh rat plasma at a concentration of 2.0 μg/ml, and the mixture was incubated at 37°C. At intervals, the samples were centrifuged and both the pellets and supernatants were assayed for amphotericin B. The profile for the recovery of amphotericin B...
from ABLC in plasma supernatant with time was very similar to that in Fig. 1. The percentage of amphotericin B from ABLC recovered in the supernatant increased with time, from 2.0% at time zero to 64.3% at 3 h. In comparison, the concentration of amphotericin B from AmB-d recovered in the supernatant remained constant during 3 h of incubation except immediately after spiking, when it was possible to pellet approximately 60% of the added amphotericin B. This may be due to the fact that the deoxycholate disperses very rapidly in plasma, allowing the insoluble amphotericin B to precipitate briefly before binding to plasma proteins (9).

The aggregation state of amphotericin B in saline can be detected by absorbance spectroscopy (12). However, this method was not suitable for plasma samples due to the high background absorbance of plasma constituents. Thus, we used CD spectroscopy to confirm the aggregated nature of amphotericin B in complexes that sedimented in ABLC-spiked plasma. The CD spectra for amphotericin B in the pellets of plasma spiked with ABLC after 0 or 180 min. of incubation are presented in Fig. 2. Immediately after spiking, the amphotericin B in the ABLC pellet had a major positive peak at 325 nm and four major negative peaks at approximately 356, 370, 390, and 420 nm. A similar spectrum was obtained with ABLC-spiked plasma that had not been centrifuged (data not shown). These spectral features are similar to those observed for authentic ABLC in saline (21). After incubation of ABLC-spiked plasma at 37°C for 180 min, the spectra of amphotericin B in the pellet showed slight alterations but still retained the general characteristics of aggregated amphotericin B. The CD spectra of the amphotericin B in the supernatant of incubated samples spiked with ABLC or samples spiked with AmB-d (data not shown) did not show features of complexed amphotericin B. These data suggest that the amphotericin B that sediments during low-force centrifugation of plasma spiked with ABLC is complexed amphotericin B (that is, still complexed with DMPC and/or DMPG), whereas that found in the supernatant is released amphotericin B.

Behavior of ABLC in vivo. After the administration of a single, intravenous bolus dose of 1 mg/kg, the mean maximum observed total amphotericin B concentration in whole blood of rats that received AmB-d was 1.13 \( \mu \)g/ml, while that for animals that received ABLC was 0.37 \( \mu \)g/ml at 5 min postdosing. Total amphotericin B in whole blood was consistently higher for the AmB-d group than for the ABLC group during the 24 h after dosing (Fig. 3). For most rats that received ABLC, amphotericin B levels in whole blood were below the level of detection by 3 h after administration of the dose. However, if only those rats with detectable levels of amphotericin B in blood are considered, the mean AUC from 0 to 24 h for AmB-d was equal to 3.41 \( \pm \) 0.91 \( \mu \)g \( \cdot \) ml/h, while that for ABLC was fourfold lower (0.85 \( \pm \) 0.41 \( \mu \)g \( \cdot \) ml/h). Although the low levels of total amphotericin B in rats that received 1 mg of ABLC per kg precluded a rigorous pharmacokinetic evaluation, examination of Fig. 3 suggests that the amphotericin B from ABLC following ABLC administration distributes more rapidly and extensively than that from AmB-d following AmB-d administration.

The ratio of the amphotericin B concentration in the plasma supernatant and in whole blood from rats that received AmB-d

![FIG. 1. Effect of time, temperature, and incubation medium on the appearance of amphotericin B in the supernatant from plasma or saline spiked with 1.00 \( \mu \)g of ABLC per ml.](image1)

![FIG. 2. CD spectra for amphotericin B in the plasma pellet obtained after incubation of 2.00 \( \mu \)g of ABLC per ml for 0 and 180 min at 37°C.](image2)

**TABLE 1.** Effect of concentration on recovery of amphotericin B in the pellet or supernatant of rat plasma spiked with ABLC

<table>
<thead>
<tr>
<th>Concen (( \mu )g/ml)</th>
<th>Recovery of amphotericin B (% of total)</th>
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<tbody>
<tr>
<td></td>
<td>Supernatant</td>
</tr>
<tr>
<td>0.3125</td>
<td>34.2</td>
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<tr>
<td>0.6250</td>
<td>31.4</td>
</tr>
<tr>
<td>1.250</td>
<td>25.8</td>
</tr>
<tr>
<td>5.000</td>
<td>18.0</td>
</tr>
<tr>
<td>10.00</td>
<td>12.8</td>
</tr>
</tbody>
</table>

* Rat plasma samples were spiked with ABLC and were incubated at 37°C for 30 min prior to separation of the pellet and supernatant.

**TABLE 2.** Effect of time on the recovery of amphotericin B in plasma spiked with ABLC or AmB-d

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Recovery of amphotericin B (% of total)</th>
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<tbody>
<tr>
<td></td>
<td>ABLC</td>
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<tr>
<td></td>
<td>Supernatant</td>
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<tr>
<td>0</td>
<td>2.0</td>
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<tr>
<td>30</td>
<td>37.9</td>
</tr>
<tr>
<td>60</td>
<td>43.3</td>
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<tr>
<td>180</td>
<td>64.3</td>
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* Fresh rat plasma samples were spiked with 2.00 \( \mu \)g of amphotericin B as ABLC or AmB-d per ml and were incubated at 37°C.
was fairly constant for all time points studied. In contrast, the ratio of amphotericin B in the supernatant to amphotericin B in whole blood for animals that received ABLC was low at the earliest time point (5 min) and increased to ratios comparable to those for AmB-d-treated animals at time points after 1 h. The AUC for amphotericin B in the plasma supernatant of rats that received ABLC could only be calculated for up to 3 h after dosing since only two rats had detectable levels at later time points. The AUC from 0 to 3 h for amphotericin B in the supernatants for rats that received ABLC was eightfold lower than that for blood from rats receiving AmB-d ($P < 0.05$).

Association of amphotericin B from ABLC or AmB-d with plasma proteins. A single pool of fresh rat plasma with a total cholesterol concentration of 0.71 mg/ml and a total protein concentration of 56.4 mg/ml was used for the in vitro studies. The plasma was spiked with AmB-d or ABLC, and the mixture was incubated for 0 or 180 min at 37°C. The proportions of amphotericin B from ABLC in the plasma supernatant at 0% and 180 min of incubation were 14.5 and 56.4%, respectively. In comparison, for AmB-d, 69.3 and 89.6% of the amphotericin B was recovered in the plasma supernatant at these respective times. At 0 min, amphotericin B from ABLC in the plasma supernatant was associated primarily with the HDL fraction and other proteins, whereas amphotericin B from AmB-d was associated primarily with the VLDL fraction and other proteins (Fig. 4). The percentage of amphotericin B from ABLC in the HDL fraction decreased with time, compared to an increase in the VLDL fraction for amphotericin B from AmB-d. The cholesterol and protein concentrations in each of the lipoprotein fractions was comparable in the ABLC-spiked and AmB-d-spiked samples (data not shown).

Groups of six rats each were treated daily with maximum tolerated doses of AmB-d (0.5 mg/kg/day) or ABLC (10 mg/kg/day). The mean amphotericin B concentrations in whole blood and plasma supernatants for these animals at 15 and 180 min after administration of the last of 15 daily doses are presented in Fig. 5. The mean concentrations of amphotericin B in whole blood for the groups given ABLC were 1.761 μg/ml at 15 min postdosing and 0.74 μg/ml at 180 min postdosing. The corresponding values for the groups given AmB-d were 0.35 and 0.21 μg/ml. Comparison of the concentrations of amphotericin B in pellets and supernatants as a function of time in plasma samples obtained from rats dosed for 15 days with ABLC (10 mg/kg; ■) or AmB-d (0.5 mg/kg; □) per ml. The recovery of amphotericin B is expressed as the percentage of total amphotericin B in the plasma supernatant accounted for in each plasma protein fraction. (A) Distribution at 0 min. (B) Distribution at 180 min.

FIG. 3. Mean blood concentration-time profiles for amphotericin B administered as either ABLC or AmB-d at 1 mg/kg to Sprague-Dawley rats. Values below the limit of quantitation were not included in the calculation of means. There were seven rats per group initially. The mean AUC from 0 to 24 h for amphotericin B for blood from rats receiving AmB-d was significantly greater than that for blood from rats receiving ABLC ($P < 0.05$).
tericin B in whole blood with those in plasma supernatants for the groups receiving ABLC suggests that approximately 60% of the amphotericin B from ABLC is complexed at both time points. In contrast, amphotericin B in whole blood and plasma supernatants for the groups receiving AmB-d were similar at the two time points. Although the dose of amphotericin B given as ABLC was 20 times greater than that of AmB-d, the amphotericin B concentrations in the plasma supernatant were only approximately one to two times those observed in the AmB-d-treated animals, a difference that was not statistically significant.

There was no significant difference in the distribution of the amphotericin B that was recovered in the plasma supernatant from samples collected from both ABLC- and AmB-d-dosed rats at both time points studied (Fig. 6). Most of the drug was recovered with nonlipoprotein or other proteins, while the rest was distributed fairly evenly among the VLDL, LDL, and HDL fractions. At 180 min after administration of the last dose, both the relative distribution and the actual amount of amphotericin B in each of the plasma supernatant protein fractions were essentially identical for ABLC- and AmB-d-dosed rats. It has been shown that 4 to 10 molecules of cholesterol in LDLs bind to 1 molecule of amphotericin B (5). The cholesterol-to-amphotericin B mole ratios in all of the plasma fractions from animals receiving ABLC or AmB-d were greater than 1,500 to 1. Thus, it is unlikely that cholesterol binding sites were saturated in any of the plasma protein fractions.

**DISCUSSION**

Differential centrifugation techniques have been used in an attempt to characterize the nature of ABLC in the circulation and the interaction(s) between ABLC and plasma protein constituents. We found that the relatively dense, complexed amphotericin B in ABLC could be sedimented in plasma or whole blood by low-speed centrifugation. The amphotericin B that sedimented in plasma spiked with ABLC had a CD spectrum characteristic of aggregated amphotericin B, as seen with authentic ABLC (21), whereas amphotericin B in the supernatants of these samples did not. A portion of the amphotericin B from plasma samples spiked with AmB-d also sedimented immediately after spiking but did not have CD spectral characteristics of amphotericin B aggregates and did not pellet at later time points. Other studies have shown that up to about 50% of amphotericin B as AmB-d added to human serum can be removed by filtration through a 0.22-μm-pore-size filter 1 min after addition to serum (presumably due to the colloidal nature of the drug in aqueous systems) but that after 5 min, greater than 85% of the drug is filterable (9).

It is known that amphotericin B interacts with erythrocytes, and it is possible that some of the amphotericin B in whole-blood samples containing ABLC sediments because it is bound to erythrocyte membranes. However, we have shown that the amphotericin B in ABLC sediments in plasma to the same degree that it sediments in whole blood. In addition, in vitro studies have shown that ABLC is approximately 100-fold less hemolytic than AmB-d to erythrocytes in vitro (21), suggesting that the complex inhibits interaction of amphotericin B with erythrocytes.

Incubation of dilute ABLC in saline at 25 or 37°C did not change its sedimentation or CD spectral characteristics, indicating that the complex remained intact under these conditions. Incubation of ABLC in blood or plasma (at concentrations typically achieved in vivo) at 25 or 37°C in vitro resulted in the gradual remodeling of the complex. The release of amphotericin B from ABLC in plasma occurred in a time-, temperature-, and concentration-dependent fashion, suggesting that saturable plasma protein interactions may play a role in the remodeling process. It is clear from these results that the use of plasma or serum for the determination of amphotericin B pharmacokinetics is likely to underestimate the total circulating amphotericin B concentrations in subjects receiving ABLC.

The levels of antimicrobial agents in serum or blood are easily measured, but efficacy is more likely dependent on the concentration in tissue (16). Administration of ABLC produces higher levels in tissue and lower circulating levels of total amphotericin B than administration of the same dose of AmB-d. We found that the AUC from 0 to 24 h for total amphotericin B in whole blood in rats administered a single 1-mg/kg intravenous bolus dose of ABLC was fourfold lower than that found in rats given 1 mg of AmB-d per kg. Similarly, in rats given repeated, daily doses of ABLC, the total amphotericin B concentration in blood was only 3.5- to 5-fold greater than that in the blood of rats given AmB-d, even though the dose of ABLC was 20 times greater than the dose of AmB-d. This is consistent with previous work with ABLC with animals (17) and humans (10, 14) and most likely is a consequence of the rapid uptake of the intact ABLC by tissues.

Comparison of the concentrations of amphotericin B in whole blood and plasma supernatant at intervals after the
administration of 1 mg of ABLC per kg indicated that complexed amphotericin B is probably present in the circulation for about 1 h after the administration of a single bolus dose. The low levels of amphotericin B remaining in the circulation after about 1 h appear to be uncomplexed or released amphotericin B.

When rat plasma spiked with ABLC was incubated at 37°C for 0 or 3 h, most of the amphotericin B was associated with the VLDL fraction at both time points. It is important, however, that a large proportion (>50%) of the amphotericin B from samples spiked with ABLC or AmB-d was associated with the nonlipoprotein or other protein fraction. This is not surprising given the fact that amphotericin B can bind to other proteins and that the concentration of nonlipoprotein proteins is much greater than that of lipoproteins in plasma. This is also consistent with data from Brajtberg et al. (5), who showed that only approximately 25% of the amphotericin B added to normal human plasma in vitro migrated with the lipoprotein fraction when this fraction was separated from the other plasma proteins by sequential ultracentrifugation. Serum nonlipoprotein proteins were not separated from the lipoprotein fractions in the studies conducted by Wasan et al. (24) that lead to the hypothesis that differences in plasma lipoprotein distribution were a major factor in the reduced renal toxicity of their liposomal preparation. Because of the dynamic nature of these endogenous carrier systems and the probable involvement of organs and tissues as well as plasma in the remodeling of ABLC, we compared the plasma protein distribution of released amphotericin B from ABLC to that obtained from AmB-d under in vivo steady-state conditions. In this case, the distribution of amphotericin B among the various plasma protein fractions in rats treated with ABLC or AmB-d did not show significant differences. Thus, it is unlikely that preferential distribution into the HDL fraction of serum plays a role in the reduced toxicity of ABLC.

Consistent with the high levels in tissue that have been observed with ABLC, we have confirmed that intravenous administration of ABLC results in much lower concentrations of circulating total amphotericin B than administration of a comparable dose of conventional amphotericin B (AmB-d). In addition, complexed amphotericin B can be separated from released amphotericin B in plasma or blood on the basis of density, and the complexes sedimented in plasma retain the unique CD spectral characteristics of aggregated amphotericin B. A substantial portion of the total amphotericin B circulating in animals given single or multiple doses of ABLC is in the form of intact complexes at early time points after dosing. It is likely that it is this fraction that is taken up by the reticuloendothelial system and that may gain access to sites where the vascular endothelium is disrupted (e.g., sites of inflammation or infection). Release of active amphotericin B might then occur within the tissues as a result of fungal or host-derived phospholipases or phagocytic digestion. The decrease in nephrotoxicity seen with ABLC may be a result of an inability of the amphotericin B in the intact complex to gain access to the target cells in the normal kidney. When the kidney itself is infected (as in renal candidiasis, for instance), the capillary endothelium may be disrupted such that intact complexes could escape from the blood into the tissues. When active amphotericin B is released within the infected tissue, it would preferentially interact with fungal cell membranes rather than host cell membranes due to its intrinsically higher affinity for ergosterol than for cholesterol. Plasma constituents may play a role in the intravascular remodeling of ABLC which results in the redistribution of amphotericin B from the complexes to plasma proteins. However, this process appears to be slow relative to the length of the processes responsible for the removal of the intact complex from the blood in vivo. The amphotericin B that remains in the circulation is gradually redistributed to the same plasma lipoprotein and nonlipoprotein fractions as amphotericin B from AmB-d, and thus, its in vivo fate is likely to be similar. However, the actual amount of amphotericin B (as a percentage of the administered dose) circulating in this form is relatively small. The high level of uptake by tissue and the low circulating levels of released amphotericin B are consistent with the concept that the enhanced therapeutic index of ABLC is due to the ability of the complex to prevent release of the drug in normal tissues and to selectively target the release of active amphotericin B at sites of fungal infection in the tissues.

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REFERENCES


