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Concentrations in Serum and Distribution in Tissue of Free and Liposomal Amphotericin B in Rats during Continuous Intralipid Infusion

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The influences of Intralipid (IL) and 0.45% normal-saline infusions on the concentration in serum and distribution in tissue of amphotericin B (AmpB) and liposomal amphotericin B (L-AmpB) in rats were compared. In animals receiving a continuous IL infusion, concentrations of AmpB in kidneys and lungs were significantly higher, but the concentration of AmpB in serum was significantly lower in animals administered AmpB versus those given L-AmpB. In animals receiving a continuous normal-saline infusion concentrations of AmpB in kidneys and the spleen were significantly higher, but the concentration of AmpB in serum was significantly lower in animals administered AmpB versus those given L-AmpB. These results suggest that the increased total serum cholesterol and high-density lipoprotein cholesterol during the IL infusion decreased the clearance of AmpB from the bloodstream and decreased the L-AmpB concentration in the kidney and lung.

Amphotericin B (AmpB) remains one of the most effective and widely used agents in the treatment of systemic fungal infections; however, its use is limited by dose-dependent nephrotoxicity (3). When AmpB is incorporated into liposomes composed of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) (7:3, wt/wt) with a lipid-to-drug ratio of 10:1, wt/wt, it is as effective as, but less toxic than, Fungizone (which consists of AmpB and desoxycholate in a 5:4, wt/wt, ratio) in experimental animal models (9, 10) and patients (8) with systemic fungal infections. However, the mechanisms that result in the enhanced therapeutic index of liposomal AmpB (L-AmpB) are not fully understood.

Previous pharmacokinetic and tissue distribution studies have described AmpB's disposition in the body as having a central compartment and two peripheral compartments (2). AmpB and L-AmpB have a large volume of distribution in humans as a result of high accumulation within tissues, and AmpB has a long terminal half-life, 15 days (2), probably due to the slow release of the drug from tissue sites.

We have demonstrated a smaller area under the serum AmpB concentration-time curve, greater volume of distribution at steady state, and faster systemic clearance of AmpB in an induced diabetic murine model which indirectly results in hyperlipidemia and hyperlipoproteinemia versus normolipidemic rats (20). Furthermore, we observed a lower distribution of AmpB in kidney and liver tissues and smaller nephrotoxic effects of AmpB in diabetic rats. However, the pharmacokinetics, tissue distribution, and extents of nephrotoxic effects of L-AmpB in diabetic rats were unchanged (20). In addition, we have demonstrated that AmpB associates predominantly with high-density lipoproteins (HDL) following a 1-h incubation at 37°C in human serum, and the amount of AmpB associated with HDL increases when AmpB is incorporated into negatively charged liposomes composed of DMPC and DMPG (17).

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Intralipid administered as a nutritional supplement in debilitated patients is regulated to prevent the development of severe hypertriglyceridemia but may increase plasma HDL levels (16). Tashiro and coworkers have demonstrated that intravenous (i.v.) fat emulsions are rapidly hydrolyzed by lipoprotein lipase and the exogenously supplied phospholipids and cholesterol accumulate primarily into low-density lipoproteins (LDL) (15). We have demonstrated with rats that a continuous infusion of 5% Intralipid for 5 days results in an increase in total serum cholesterol and HDL cholesterol without altering LDL cholesterol (18) or total serum triglycerides.

Moreau and coworkers have shown that when AmpB was mixed with Intralipid in neutropenic patients, renal toxicity was reduced without altering of the total sodium intake (11); however, Joly et al. have demonstrated that deoxycholate AmpB associated with Intralipid in the treatment of cryptococcal meningitis patients with AIDS (6) showed no differences in nephrotoxicity between AmpB and AmpB infused with Intralipid. Kirsh and coworkers further reported that the formulation of an emulsion of AmpB and 20% Intralipid reduced acute AmpB toxicity in the treatment of systemic murine candidiasis, without altering its antifungal activity (7). Since AmpB associates predominantly with HDL in serum following 1 h of incubation and the continuous infusion of Intralipid increases total serum cholesterol and HDL cholesterol, it is possible that AmpB's interactions with HDL increase in the presence of a continuous Intralipid infusion. The present studies examined the influence of a concurrent Intralipid infusion on the clearance from the bloodstream and distribution of AmpB and L-AmpB in tissue in rats 4 h after a single i.v. bolus dose.

A total of 12 male Fischer rats (250 to 300 g) (Harlan Breeders, Indianapolis, Ind.) were anesthetized with pentobarbital sodium (Nembutal) (50 mg/kg of body weight intraperitoneally), and a central venous catheter was inserted via the jugular vein for total parental nutrition (18). Rats were individually housed in stainless steel metabolism cages modified to accommodate the infusion apparatus in a 12-h dark-light cycle animal facility with controlled temperature and humidity. The

TABLE 1. Cholesterol levels in rats receiving a continuous infusion of 0.45% NS or 5% Intralipid for 6 days at a flow rate of 1.2 ml/h

Treatment	Cholesterol (mg/dl)[a]			HDL/LDL cholesterol ratio
	Total	HDL	LDL	
NS	34.3 ± 4.2	13.1 ± 2.8	8.1 ± 4.2	1.62 ± 0.7
Intralipid	68.7 ± 7.1[b]	25.7 ± 1.9[b]	11.9 ± 1.0	2.16 ± 0.5

[a] Mean ± standard deviation.
[b] Significantly different from the corresponding value for NS ($P < 0.05$).

animals were allowed to recover for 48 h after the surgical procedure. Water and rat chow (Ralston Purina Co., St. Louis, Mo.) amounts were unrestricted throughout the study, and 0.45% normal saline (NS) was infused to keep the infusion line patent during the study. Animal food intake was determined daily.

Rats (n = 12) were randomly chosen and received a continuous infusion of either 0.45% NS (n = 6) or 5% Intralipid (n = 6) continuously for 7 days at a rate of 1.2 ml/h. After 6 days, whole blood samples (1 ml) were removed through the catheter and the blood was centrifuged for 5 min at 13,000 × g to obtain the serum (0.5 ml). This serum was separated into its HDL and LDL fractions by size exclusion and affinity chromatography (17, 18), and total, HDL, and LDL cholesterol concentrations were determined to confirm that the Intralipid infusion had elevated the total serum cholesterol and HDL cholesterol as previously described (18).

On day 7 of the Intralipid and NS infusions the animals were anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally) and the femoral vein and artery were catheterized. During a continuous infusion of Intralipid (n = 3) or 0.45% NS (n = 3) some rats received a single i.v. dose, via the femoral vein, of AmpB (Fungizone; Bristol-Myers Squibb; 1.0 mg/kg); other rats receiving a continuous infusion of Intralipid (n = 3) or 0.45% NS (n = 3) received a single i.v. dose, via the femoral vein, of L-AmpB (1.0 mg/kg). L-AmpB composed of DMPC and DMPG was prepared as previously described (20). Whole blood samples (1.0 ml) were obtained through the femoral artery 240 min after the i.v. bolus dose. Blood samples were collected in drug-free microcentrifuge tubes, clotted on ice, and centrifuged; serum was stored at −20°C until analysis. Following the 240-min blood sampling each animal was humanely sacrificed with pentobarbital sodium (300 mg/kg intraperitoneally) and the liver, right kidney, and lung were removed, dried, and weighed. Each organ was stored at −20°C until analysis.

Sera from single blood samples obtained on day 6 of the infusion were used for the determination of total cholesterol, HDL cholesterol, and LDL cholesterol. Cholesterol was measured by standard enzymatic reaction (Sigma Chemical Co., St. Louis, Mo.) (18). AmpB levels in serum and organ tissues were analyzed by high-pressure liquid chromatography (HPLC) as previously described (20). Briefly, serum samples (100 μl) were mixed with equal volumes of methanol, which was vortexed for 10 s and centrifuged (13,000 × g for 2 min). The extractant (75 μl) was analyzed in comparison with an external standard calibration curve by an HPLC method. Tissue samples (0.5 g) were homogenized with 1.0 ml of methanol for 3 min, and the extractant was analyzed by HPLC. Control organ tissues mixed with known amounts of AmpB stock solution were used to establish standard curves. The sensitivity of this assay was 50 ng/ml, with an intraday coefficient of variation of 5% (linear range, 50 to 5,000 ng/ml; r^2 = 0.99). Significant differences in serum AmpB, tissue AmpB, and cholesterol concentrations between groups were determined by Student's independent t test. A difference was considered significant when P was <0.05. All data are presented as means ± standard deviations.

For all studies of AmpB and L-AmpB concentrations in serum and distribution in tissue, total cholesterol and HDL cholesterol were significantly elevated in animals receiving Intralipid versus those receiving NS on the 6th day of the infusions (Table 1). When animals were administered a single dose of AmpB, there were no significant differences between the distribution of AmpB in tissue after a continuous Intralipid infusion and its distribution after a continuous NS infusion (Table 2). However, serum AmpB concentrations in animals receiving a continuous Intralipid infusion were significantly higher than those in animals receiving the NS infusion (Table 2).

In animals administered a single dose of L-AmpB, the lowest levels of AmpB were found in the kidney and lung tissues with both the Intralipid infusion and the NS infusion (Table 2). The concentrations of AmpB in serum in animals administered a single dose of L-AmpB while receiving a continuous Intralipid infusion were significantly higher than those in animals receiving a continuous NS infusion (Table 2). No significant differences in liver uptake between any of the groups were observed; however, the levels of spleen uptake in animals administered L-AmpB were significantly lower than those in animals administered free AmpB, and the levels in animals receiving the Intralipid infusion were significantly lower than those in animals receiving the NS infusion (Table 2).

The administration of AmpB resulted in a higher concentration of AmpB in serum without altering AmpB's distribution in tissue in animals receiving Intralipid than the concentration in animals receiving the NS infusion, suggesting that AmpB remains longer in the systemic circulation. The admin-

TABLE 2. Distribution in tissues and concentrations in serum of AmpB and L-AmpB (1.0 mg/kg) in rats receiving a continuous infusion of 0.45% NS or 5% Intralipid for 7 days[a]

Tissue or serum	AmpB		L-AmpB	
	NS	Intralipid	NS	Intralipid
Kidney	735.1 ± 285.6	657.8 ± 210	298.2 ± 96.2[b]	155.9 ± 56.3[b,c]
Liver	468.2 ± 169.2	587 ± 305.5	398.0 ± 280.4	667.9 ± 665.3
Lung	1,906.4 ± 1,526.8	1,799.3 ± 1,605	695.3 ± 250.2	160.1 ± 46.2[b,c]
Spleen	2,112.9 ± 403.4	2,242.5 ± 1,445	906.6 ± 52.6[b]	1,489 ± 419.9[c]
Serum	70.01 ± 13.7	139.3 ± 15.4[d]	183.7 ± 36.9[d]	304.7 ± 20.3[b,c,d]

[a] The data are means ± standard deviations (n = 3) in nanograms of AmpB per gram of tissue or per milliliter of serum.
[b] Significantly different from the corresponding value for AmpB with Intralipid ($P < 0.05$).
[c] Significantly different from the corresponding value for L-AmpB with NS ($P < 0.05$).
[d] Significantly different from the corresponding value for AmpB with NS ($P < 0.05$).

istration of Intralipid appears not to alter the elimination rates of apolipoprotein AI (protein component of HDL) and HDL from the bloodstream (5) but increases serum HDL (18), partly because of an increase in hepatic protein synthesis of apolipoprotein AI and AII (4). Furthermore, since the administration of Intralipid may also increase the activity of lecithin-cholesterol acyltransferase activity (1), an enzyme which converts free cholesterol into cholesteryl esters (cholesteryl esters are then taken up by HDL), the increased HDL cholesterol and HDL cholesterol/LDL cholesterol ratio may be due to this increase in lecithin-cholesterol acyltransferase activity and the absence in the rat of lipid transfer protein (13), an enzyme responsible for the transfer of cholesteryl esters between HDL and LDL (12). The increase in HDL cholesterol without altering of the LDL cholesterol may increase the interaction of AmpB with the cholesterol and cholesteryl esters of HDL and contribute to the observed decreased clearance of AmpB from the bloodstream. However, since the half-life of HDL cholesteryl esters in the rat in the bloodstream is 4 h (14), measuring tissue AmpB concentrations 4 h after i.v. administration of AmpB may be too early to see changes in the distribution of AmpB in tissue.

Animals given Intralipid and L-AmpB had lower AmpB concentrations in the kidney and lung tissues but a higher AmpB concentration in the spleen than the concentrations in animals given NS and L-AmpB. Animals receiving NS and L-AmpB had lower concentrations of AmpB in the kidney than the concentrations in animals receiving NS and AmpB. Since L-AmpB associates predominantly with HDL (17), the reduced AmpB concentration in the kidney tissues of animals receiving the Intralipid or NS infusion may be due to the low expression of HDL receptors in the kidney (19).

Our results suggest that AmpB's association with HDL may be responsible for the decreased clearance of the drug from the blood. Increasing AmpB's association with HDL by incorporating the drug into negatively charged liposomes (L-AmpB) further reduced the amount of AmpB in the kidney and may be responsible for the decreased renal toxicity associated with the i.v. administration of L-AmpB.

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