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Amphotericin B Lipid Complex or Amphotericin B Multiple-Dose Administration to Rabbits with Elevated Plasma Cholesterol Levels: Pharmacokinetics in Plasma and Blood, Plasma Lipoprotein Levels, Distribution in Tissues, and Renal Toxicities

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The purpose of the present study was to determine if a relationship exists between the plasma cholesterol concentration, the severity of amphotericin B (AmpB)-induced renal toxicity, and the pharmacokinetics of AmpB in plasma in hypercholesterolemic rabbits administered multiple doses of amphotericin B (AmB) deoxycholate (Doc-AmB) and AmB lipid complex (ABLC). After 7 days of administration of a cholesterol-enriched diet (0.50% [wt/vol]) or a regular rabbit diet, each rabbit was administered a single intravenous bolus of Doc-AmB ($n = 8$) or ABLC ($n = 10$) (1.0 mg/kg of body weight) daily for 7 consecutive days (a total of eight doses). Blood samples were obtained daily before and 24 h after the administration of each dose and serially thereafter following the administration of the last dose for the assessment of pharmacokinetics in plasma, kidney toxicity, plasma lipoprotein levels, and drug distribution in tissue. The pharmacokinetics of AmB in blood following the administration of ABLC were also determined in rabbits fed cholesterol-enriched and regular diets ($n = 3$ each group). Before drug treatment, cholesterol-fed rabbits demonstrated marked increases in total, low-density lipoprotein (LDL), and triglyceride-rich lipoprotein (TRL) cholesterol levels in plasma compared with the levels in rabbits on a regular diet. No significant differences in total plasma triglyceride levels were observed. Significant increases in plasma creatinine levels were observed in rabbits fed a cholesterol-enriched diet ($P < 0.05$) and rabbits fed a regular diet ($P < 0.05$) when administered AmB. However, the magnitude of this increase was twofold greater in rabbits fed a regular diet than in rabbits fed a cholesterol-enriched diet. An increase in plasma creatinine levels was observed only in rabbits on a cholesterol-enriched diet administered ABLC. The pharmacokinetics of AmB were significantly altered in rabbits on a cholesterol-enriched diet administered Doc-AmB or ABLC compared to those in rabbits on a regular diet administered each of these compounds. The pharmacokinetics of AmB in blood were significantly different following ABLC administration but not following Doc-AmB administration in both rabbits fed cholesterol-enriched diets and rabbits fed regular diets compared to their corresponding pharmacokinetics in plasma. An increased percentage of AmB was recovered in the TRL fraction when Doc-AmB was administered to rabbits fed a cholesterol-enriched diet than when it was administered to rabbits fed a regular diet. Furthermore, an increased percentage of AmB was recovered in the LDL and TRL fractions when ABLC was administered to rabbits fed a cholesterol-enriched diet rabbits fed a regular diet. These findings suggest that an increase in plasma cholesterol levels modifies the pharmacokinetics of AmB and renal toxicity following the administration of multiple intravenous doses of Doc-AmB and ABLC.

Disseminated fungal infections such as candidiasis, histoplasmosis, and aspergillosis are on the rise, particularly in patients with cancer, organ transplant recipients, diabetics patients, and patients with AIDS (4, 25). Among these patients invasive fungal infections may account for as many as 30% of deaths (4, 39). Despite the development of a number of new antifungal agents (10), amphotericin B (AmB) formulated as a micelle suspension with deoxycholate (Doc-AmB) remains one of the most effective agents in the treatment of systemic fungal infections (18). However, Doc-AmB use is often limited by the development of kidney toxicity manifested by renal vasoconstriction with a significant decrease in the glomerular filtration rate and renal plasma flow and by renal potassium and magnesium wasting (10, 18, 39).

Incorporation of many drugs, including chemotherapeutic and antifungal agents, into liposomes minimizes toxicity without a loss of the pharmacological effect (2, 15, 22, 29). In addition, when AmB was complexed with lipid to form AmB lipid complex (ABLC), AmB was selectively taken up by mononuclear phagocytes and delivered principally to the liver and the lung (16, 28). Survival of mice infected with *Histoplasma capsulatum* was greater with ABLC than with AmB treatment, in part due to the higher concentrations of AmB in liver and lung

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tissue (28). Moreover, ABLC was less toxic for these animals than it was for infected mice administered equivalent amounts of Doc-AmB. Recent studies by Bhamra et al. (3) have suggested that the very low levels of circulating protein-bound AmB that they observed after administration of ABLC to rats was a result of rapid tissue uptake, which led to reduced toxicity.

Doc-AmB and ABLC are examples of drug formulations that can associate with lipoproteins in serum and plasma in vivo and in vitro (32–34, 37, 39). We believe that this property has a major effect on the efficacy and safety of these compounds since they are often administered to patients with abnormal cholesterol metabolism (8, 9, 12). Disease-related changes in liver and kidney function and blood flow may also alter the pharmacokinetics and toxic effects of these drugs. However, it is our contention that understanding of the mechanisms by which dyslipidemia (abnormal serum lipid concentrations) affects the actions of these compounds is essential prior to Doc-AmB and ABLC administration.

There is growing evidence that supports our hypothesis that increases in serum cholesterol concentrations increase the renal toxicity of Doc-AmB. Specifically, we have previously observed that when Doc-AmB is administered to hypercholesterolemic, insulin-dependent diabetic rats, the magnitude of nephrotoxicity was greater than that in control nondiabetic rats. Furthermore, the half-life and volume of distribution of AmB in serum were increased in diabetic rats compared to those in nondiabetic control rats (35). Preliminary studies recently completed by our laboratory have shown that upon administration of a single dose to rabbits fed a cholesterol-enriched diet (cholesterol-fed rabbits), Doc-AmB was more nephrotoxic than when it was administered to control rabbits (39). The enhanced nephrotoxicity of AmB is probably mediated through drug binding to the low-density lipoprotein (LDL) receptor (34, 37). Furthermore, recent studies with kidney cells have also shown that when the numbers of LDL receptors expressed on these cells were reduced, the AmB that bound to LDL was less toxic than unbound AmB (37). These findings suggest that increases in the levels of AmB binding to LDL in serum enhance the ability of AmB to damage kidney cells.

However, unlike Doc-AmB, an increase in serum cholesterol concentrations does not affect the pharmacokinetics or modify the renal toxic effects of AmB following the administration of a single intravenous dose of ABLC (39). Specifically, we have previously observed that when ABLC was administered to hypercholesterolemic insulin-dependent rats, the pharmacokinetics and renal toxic effects of AmB were not markedly altered compared to those in nondiabetic rats (35). Furthermore, it has been suggested that the renal toxicity of ABLC bound to lipoproteins in serum may differ from that of AmB alone. Whereas AmB alone binds preferentially to LDL and can be internalized into renal cells that express LDL receptors, resulting in toxicity (33), ABLC predominantly binds to high-density lipoprotein (HDL) (34), remains in the bloodstream, and lacks toxicity. In addition, our preliminary findings suggest that AmB bound to HDL is less toxic to kidney cells than AmB bound to LDL, possibly due to the small number of HDL receptors present on these cells (32). Taken together, these findings suggest that the decreases in the levels of binding of AmB to LDL in serum by incorporation of the drug into a phospholipid vesicle (ABLC) diminish the ability of AmB to damage kidney cells.

These studies provide compelling evidence that serum or plasma lipoprotein levels have a major effect on the toxicity and pharmacokinetics of AmB formulations. However, one cannot be sure that these observations were a direct result of variations in the serum or plasma cholesterol concentration or were due to sex differences or the disease models investigated. Furthermore, the data generated from studies with experimental rat models cannot be extrapolated to what may be observed in humans because the behaviors of lipoproteins in rats are very different from the behaviors in humans (i.e., HDLs in rats are the major carrier of cholesterol, while LDLs are the major carrier of cholesterol in humans) and the activity of a lipid transfer protein (LTP 1), a protein that is responsible for the transfer of lipids in serum among different lipoprotein subfractions (19) and that is measurable in humans, has minimal activity in rats (20, 21). In addition, the studies with animals that have been completed were done following the administration of only a single intravenous dose of either Doc-AmB or ABLC and not following the administration of a more clinically relevant multiple-dose regimen.

Therefore, the purpose of the study described here was to investigate the relationship between the serum total cholesterol level and lipoprotein cholesterol concentration, the severity of AmB-induced kidney toxicity, and the pharmacokinetics of AmB in serum in hypercholesterolemic rabbits administered multiple doses of Doc-AmB and ABLC. The study was completed in order to mimic the multiple-dose regimen of Doc-AmB and ABLC commonly used clinically in a relevant experimental animal model (i.e., rabbits, in which the behaviors of lipoproteins are similar to those in humans [20, 21]). On the basis of the results of our preliminary tissue culture studies with tissues from rabbits and humans, our working hypothesis was that an elevation in the serum cholesterol concentration increases the association of Doc-AmB with cholesterol-rich lipoproteins (predominantly, the apolipoprotein B-rich lipoproteins LDL and triglyceride-rich lipoprotein [TRL, which include very-low-density lipoproteins and chylomicrons]), resulting in increased kidney toxicity. However, increases in the serum cholesterol concentration would not modify the kidney toxicity profile of ABLC.

MATERIALS AND METHODS

AmB and ABLC formulations. AmB, which contains sodium deoxycholate (Fungizone; Doc-AmB) and which is reconstituted in sterile water, was purchased from Bristol-Myers-Squibb (Newark, N.J.). The method of preparation of lipid complexes containing AmB (ABLC; Abelcet, The Liposome Company, Princeton, N.J.) has been described previously (3, 32). These lipid complexes use nontoxic phospholipids (dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol) and are reconstituted in normal saline.

Cholesterol-fed rabbit model. All rabbits used for this study were cared for in accordance with the principles promulgated by the Canadian Council on Animal Care and the University of British Columbia. They were housed within individual metabolism cages in an animal facility with a 12-h dark–12-h light cycle and controlled temperature and humidity. Water and food (Purina Rabbit Chow 5001) were unrestricted throughout the study. All the rabbits were allowed 3 days to acclimate to their environment prior to experimentation. Female New Zealand White rabbits (weight, 3.0 to 4.0 kg; Jeo-Bet Rabbits Ltd., Aldon, British Columbia, Canada) that exhibit hypercholesterolemia (induced by a cholesterol-enriched diet, as described previously [39]) were used (see Table 1). The cholesterol-fed rabbits received Purina rabbit chow supplemented with 2.5% (wt/vol) coconut oil and 0.50% (wt/vol) cholesterol for 7 days prior to the experiment.

This was an ideal model because no kidney or liver function and hematological profile abnormalities were observed in the cholesterol-fed and age-matched New Zealand White rabbits, and 3-ml blood samples were obtained without significant changes in blood flow (20, 21). Furthermore, the rabbit was the appropriate experimental animal for these studies because the behavior and structure of rabbit lipoproteins are similar to those of human lipoproteins (7). The operative technique for insertion of a catheter for permanent placement was modified from that of Walsh and coworkers (31) and other investigations (39) to include a heparin lock device (Harvard Apparatus Canada, Saint-Laurent, Quebec, Canada).

Separation of lipoproteins in plasma. The strategy for separation of rabbit plasma samples (3.0 ml) from the 5-min blood collection into lipoprotein (HDL, LDL, and TRL [which contains chylomicrons and very-low-density lipoproteins]) and lipoprotein-deficient (LPDP) fractions was by step-gradient ultracentrifugation, as described previously (38, 39). To ensure that the lipoprotein distribution of AmB was a result of its association with each lipoprotein and not a result of the density of the formulation, the distributions of the AmB formulation reconstituted in sterile water (Fungizone; Doc-AmB) and ABLC reconstituted in normal saline within the LPDP fraction were determined. The majority (>90%) of AmB was found in the density range of >1.21 g/ml, suggesting that the AmB distribution within the ultracentrifuge tubes following incubation in rabbit plasma is not a function of the formulation density (38).

Characterization of lipoproteins. Lipoprotein preparations were characterized with respect to their lipid and protein compositions. Cholesterol (esterified and unesterified), triglyceride, and protein were quantitated by established colorimetric techniques, as described previously (35, 37).

Measurement of AmB levels. AmB levels in whole-blood, plasma, tissue, and lipoprotein fractions were analyzed by high-pressure liquid chromatography (HPLC), as described previously (35,37). Briefly, whole-blood, plasma, and lipoprotein samples (100 μl each) were mixed with equal volumes of methanol, and the mixtures were vortexed for 10 s and centrifuged (13,000 × *g* for 2 min). The extract (75 μl) was analyzed in comparison with an AmB external standard calibration curve. Tissue samples (0.5 g) were homogenized with 1.0 ml of methanol for 3 min, and the extract was analyzed by HPLC. Control organ tissues mixed with known amounts of AmB stock solutions were used to establish standard curves. The sensitivity of this assay was 5 ng/ml, with an intraday coefficient of variation of 5% (linear range, 5 to 5,000 ng/ml; $r^2 = 0.99$).

Assessment of renal function. To assess renal function, plasma creatinine concentrations prior to and 5 min following the administration of the last dose of Doc-AmB or ABLC were measured by standard enzymatic reactions (Sigma Chemical, St. Louis, Mo.). For the purposes of this study and on the basis of our preliminary studies with rats (35) and humans (33), the criteria for measurable kidney toxicity was set as a 50% increase in the serum creatinine concentration from the baseline concentration. The time of 5 min following administration of the last dose was chosen because initial studies demonstrated that, following the administration of a single intravenous dose of Doc-AmB (1 mg/kg of body weight) daily for 7 consecutive days to rabbits, the serum creatinine concentration reached its maximum level from the baseline level 5 min following administration of the last dose (data not shown).

Experimental design. Cholesterol-fed ($n = 9$) or regular diet-fed ($n = 9$) female New Zealand White rabbits (weight, 3 to 4 kg) were administered, through the jugular vein, a daily intravenous dose of Doc-AmB or ABLC (1 mg/kg) for 7 consecutive days (total of eight doses). Preliminary studies have shown that a Doc-AmB dose of 1 mg/kg is sufficient for the treatment of experimental candidiasis and yet results in measurable kidney toxicity (35–37). In addition, four cholesterol-fed and normolipidemic rabbits were administered vehicle controls (sterile water or normal saline). Serial blood samples were obtained prior to and at 0.083, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h following administration of the last dose of Doc-AmB or ABLC and were stored in centrifuge tubes. Blood samples were also obtained 24 h (trough levels) after administration of the preceeding day's dose. Plasma was harvested and stored at 4°C prior to analysis to prevent any redistribution of drug. Preliminary studies have shown that AmB does not redistribute between lipoprotein fractions at 4°C (37). After retrieval of a sample 72 h following administration of the last dose, each rabbit was humanely sacrificed (with sodium pentobarbital [60 mg/kg] administered intravenously over 2 min through the marginal ear vein) and the liver, right kidney, spleen, heart, and lung were removed, dried, and weighed. Each organ was stored at −20°C until analysis.

Preliminary investigations by Adedoyin et al. (1) have reported that the biological fluid that is used influences the characterization of the pharmacokinetics of AmB after ABLC administration but not after AmB administration. They found that when ABLC was incubated in vitro in whole blood and then centrifuged to separate the plasma, the majority of AmB was located in the pellet. This suggests that analysis of AmB in plasma following ABLC administration would underestimate the systemic concentration of the drug. Thus, following the administration of ABLC to both cholesterol-fed and regular diet-fed female rabbits, pharmacokinetic analysis of AmB was also conducted with whole blood as well as plasma.

Pharmacokinetic analysis. The pharmacokinetic parameters mean residence time (MRT), total body clearance (CL), and volume of distribution at steady state (V_{SS}) were estimated by compartmental analysis with the WINNONLIN nonlinear estimation program (26). It was concluded that the plasma (and whole blood) AmB concentration data fit a two-compartment model based on goodness-of-fit and residual-sum-of-square estimations with the WINNONLIN program and a preliminary analysis from the single-dose studies (39). In addition, an independent criterion (the Akaike information criterion) for determination of the goodness of fit was used. The concentrations of AmB in plasma (and in whole blood following ABLC administration) were plotted against time on log-linear graph paper, and the distribution phase (α) and the terminal half-life were estimated by the method of residuals (26). The area under the AmB concentration-time curve (AUC) from time zero to infinity ($AUC_{0-\infty}$) was estimated by the trapezoidal rule (26).

Statistical analysis. The pharmacokinetics of AmB in plasma and whole blood, the concentration of AmB in tissue, the distribution of AmB among the lipoproteins, the plasma creatinine concentration, and lipid levels were compared between the drug-treated and control groups of animals by analysis of variance (INSTAT2; GraphPad Inc.). Critical differences were assessed by Tukey post hoc tests. A difference was considered significant if the probability that chance would explain the results was reduced to less than 5% ($P < 0.05$). All data were expressed as the mean ± standard deviation.

RESULTS

The mean weight of the cholesterol-fed rabbits was not significantly different from that of the regular diet-fed rabbits prior to and during drug administration (data not shown). Similarly, kidney, liver, lung, spleen, and heart weights were not different between the cholesterol-fed and regular diet-fed rabbits (data not shown).

Total, LDL, and TRL cholesterol concentrations in plasma were significantly higher in cholesterol-fed rabbits than in regular diet-fed rabbits ($P < 0.05$) prior to the initiation of therapy (data not shown) and 5 min following administration of the last dose of Doc-AmB or ABLC (Table 1). However, plasma creatinine levels were not significantly different between cholesterol-fed and regular diet-fed rabbits prior to drug administration (Table 1). Significant increases in the percentages of baseline plasma creatinine levels were observed in cholesterol-fed ($P < 0.05$) and regular diet-fed rabbits ($P < 0.05$) administered Doc-AmB (Table 1). Increases in total LDL, and TRL cholesterol levels in plasma were observed in rabbits receiving a cholesterol-enriched diet (0.5% [wt/vol] cholesterol) for 7 days compared to those in rabbits receiving a regular diet, as reported previously (36) (data not shown). However, no differences in total plasma or lipoprotein triglyceride levels were observed in plasma (data not shown), as reported previously (39).

The $AUC_{0-\infty}$s for both Doc-AmB and ABLC in plasma after the administration of multiple intravenous doses to cholesterol-fed rabbits were significantly higher than the $AUC_{0-\infty}$s for Doc-AmB and ABLC in regular diet-fed rabbits ($P < 0.05$) (Fig. 1 and 2A; Table 2). $AUC_{0-\infty}$ for ABLC in the whole blood of cholesterol-fed rabbits was significantly higher than the $AUC_{0-\infty}$ of ABLC in the whole blood of regular diet-fed rabbits ($P < 0.05$) (Fig. 2B; Table 2). The α half-life was prolonged in plasma of cholesterol-fed rabbits administered ABLC compared to that in regular diet-fed rabbits (Fig. 2A; Table 2). No significant differences in the elimination (β) half-life in plasma (Fig. 2A) and whole blood (Fig. 2B) or the MRT

TABLE 1. Biochemical characteristics of plasma after administration of multiple intravenous doses of Doc-AmB and ABLC to regular diet-fed and cholesterol-fed rabbits[a]

Experimental group and drug	Plasma creatinine level			Total plasma C[c] (mg/dl)	TRL-C[c] (mg/dl)	LDL-C[c] (mg/dl)	HDL-C[c] (mg/dl)
	With regard to drug administration (μmol/liter)		% Change from baseline level				
	Prior to first dose[b]	5 min after last dose[c]					
Regular diet							
Doc-AmB	93 ± 11	252 ± 152	+171	108 ± 22	13 ± 3	44 ± 18	42 ± 1
ABLC	91 ± 11	95 ± 15[d]	+4.4	67 ± 8[d]	17 ± 3	25 ± 5	25 ± 6[d]
Cholesterol-enriched diet							
Doc-AmB	125 ± 36	230 ± 160	+84	891 ± 199[e]	667 ± 172[e]	181 ± 35[e]	34 ± 4
ABLC	111 ± 19	141 ± 44	+27	734 ± 249[e]	628 ± 234[e]	81 ± 14[d,e]	24 ± 7

[a] By these regimens the animals each received 1 mg of AmB per kg. The cholesterol enrichment contained 0.5% (wt/vol) cholesterol. Data are expressed as means ± standard deviations ($n = 5$ for ABLC for rabbits on a cholesterol-fed diet and $n = 4$ for all other treatment groups). Total plasma C, total plasma cholesterol levels; TRL-C, triglyceride-rich lipoprotein level (which includes VLDL and chylomicrons); LDL-C, LDL cholesterol level; HDL-C, HDL cholesterol level.
[b] Plasma creatinine levels prior to administration of the first Doc-AmB or ABLC dose and 7 days after regular or cholesterol-enriched diet.
[c] Refers to 5 min following administration of the last dose on day 7.
[d] $P < 0.05$ versus Doc-AmB treatment.
[e] $P < 0.05$ versus rabbits fed regular diet.

were observed (Table 2). The V_{SS} in plasma was significantly lower in cholesterol-fed rabbits than regular diet-fed rabbits administered Doc-AmB and ABLC ($P < 0.05$) (Table 2). Systemic CL from plasma was decreased in cholesterol-fed rabbits compared to that in regular diet-fed rabbits administered Doc-AmB and ABLC (Table 2). The systemic CL from whole blood was significantly decreased in cholesterol-fed rabbits compared to that in regular diet-fed rabbits administered ABLC ($P < 0.05$) (Table 2). Differences in the pharmacokinetics of AmB in whole blood and plasma were observed following ABLC administration to cholesterol-fed rabbits compared to those observed in regular diet-fed rabbits (Fig. 2A and B).

The concentrations of AmB in kidney tissue were greater in cholesterol-fed rabbits than in regular diet-fed rabbits administered ABLC (Table 3). In addition, the kidney AmB concentrations were greater in both cholesterol-fed and regular diet-fed rabbits when the rabbits were administered Doc-AmB than when they were administered ABLC (Table 3). However, no differences in liver and lung AmB concentrations were observed following the administration of Doc-AmB or ABLC to both cholesterol-fed and regular diet-fed rabbits (Table 3). Lung AmB concentrations were markedly lower after AmB administration than after ABLC administration in animals fed a regular diet (Table 3). Spleen AmB concentrations were higher in cholesterol-fed and regular diet-fed rabbits administered ABLC than in those administered AmB (Table 3). Heart AmB concentrations were significantly greater in cholesterol-fed rabbits following the administration of Doc-AmB com-

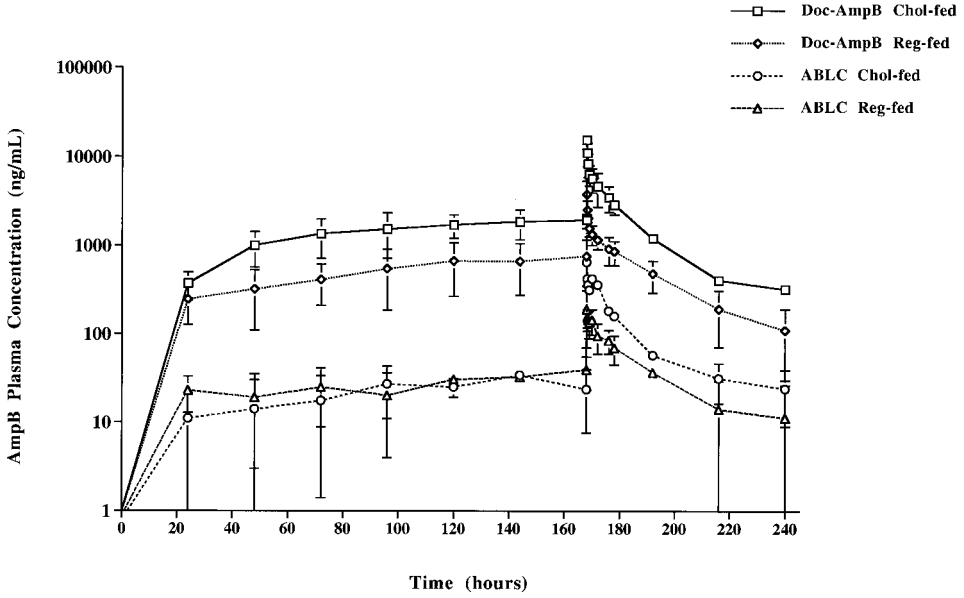

FIG. 1. Plasma AmB concentration-versus-time curve on a log-linear graph following the administration of all intravenous doses of Doc-AmB or ABLC (1 mg/kg) to cholesterol (Chol)-fed and regular diet-fed rabbits. Values are means ± standard deviations ($n = 4$ for Doc-AmB and $n = 5$ for ABLC).

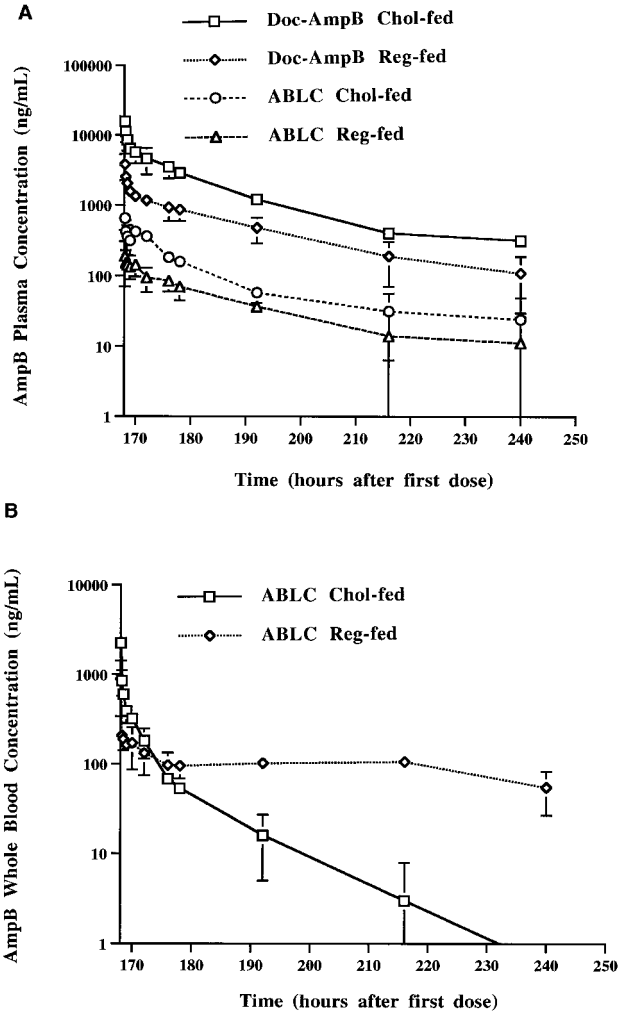

FIG. 2. (A) Plasma AmB concentration-versus-time curve on a log-linear graph following the last administration of intravenous dose of Doc-AmB or ABLC (1 mg/kg) to cholesterol (Chol)-fed and regular diet (Reg)-fed rabbits. Values are means ± standard deviations (n = 4 for Doc-AmB and n = 5 for ABLC). (B) Whole-blood AmB concentration-versus-time curve on a log-linear graph following the administration of the last intravenous dose of ABLC (1 mg of AmB/kg) to cholesterol-fed and regular diet-fed rabbits. Values are mean ± standard deviations (n = 3).

pared to those obtained following the administration of ABLC ($P < 0.05$) (Table 3).

The distribution of AmB in plasma in vivo was determined 5 min following the intravenous administration of the final doses of Doc-AmB and ABLC. Following the administration of Doc-AmB a greater percentage of AmB was recovered in the TRL fraction of cholesterol-fed rabbits than in the TRL fraction of regular diet-fed rabbits ($P < 0.05$) (Fig. 3A). However, following administration of Doc-AmB, a lower percentage of AmB was recovered in the LPDP fraction (which contains albumin and α-1-glycoprotein) of cholesterol-fed rabbits than in the LPDP fraction of regular diet-fed rabbits ($P < 0.05$) (Fig. 3A). Following the administration of ABLC, a greater percentage of AmB was recovered in the LDL and TRL fractions of cholesterol-fed rabbits than in those fractions of regular diet-fed rabbits ($P < 0.05$) (Fig. 3B). However, following the administration of ABLC, a lower percentage of AmpB was recovered in the HDL and LPDP fractions (which contains albumin and α-1-glycoprotein) of cholesterol-fed rabbits than in those fractions of regular diet-fed rabbits (Fig. 3B).

DISCUSSION

The administration of Doc-AmB has been limited by its dose-dependent kidney toxicity, but this has not been predictable by monitoring of the plasma and/or serum drug concentration (25). Many clinicians and scientists have assumed that the plasma and/or serum drug concentration is directly related to the concentration at the site of action. Error in this assumption may be due to underlying or changing disease states or altered drug-protein binding parameters. Since AmB is an example of drug that, when formulated into a lipid complex, associates with lipoproteins both in vivo and in vitro (37), we studied the influence of experimentally induced hypercholesterolemia on the disposition and toxicity of AmB following treatment of rabbits with multiple doses of Doc-AmB and ABLC.

Following the administration of multiple doses of Doc-AmB, there were considerable differences in the disposition of AmB, the distribution of AmB among lipoproteins, and the distribution of AmB in tissue in hypercholesterolemic rabbits compared to those in their normolipidemic counterparts. The $AUC_{0-\infty}$ for AmB in plasma was elevated in hypercholester-

TABLE 2. Pharmacokinetic parameters of drug in plasma and whole blood (for ABLC) after administration of multiple intravenous doses of Doc-AmB and ABLC to regular diet-fed and cholesterol-fed rabbits[a]

Experimental group and drug	$AUC_{0-\infty}$ (μg · h/ml)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	MRT (h)	V_{ss} (liters/kg)	CL (ml/h/kg)
Regular diet						
Doc-AmB (plasma)	35.3 ± 9.2	4.4 ± 1.8	101.4 ± 20.8	141 ± 26	4.4 ± 2.1	30.1 ± 8.9
ABLC (plasma)	2.6 ± 1.2[c]	0.3 ± 0.1[c]	87.1 ± 35.4[c]	126 ± 51	50 ± 9[c]	475 ± 259[c]
ABLC (whole blood)	18.1 ± 3.2[c,d]	1.8 ± 0.3[c,d]	641.6 ± 139.5[c,d]	920 ± 203[c,d]	52 ± 14[c]	56.6 ± 11[c,d]
Cholesterol-enriched diet						
Doc-AmB (plasma)	140.4 ± 32.8[b]	4.9 ± 2.3	110.3 ± 28.1	152 ± 38	1.1 ± 0.3[b]	7.5 ± 2.0[b]
ABLC (plasma)	10.0 ± 3.9[b,c]	4.3 ± 3.9[b]	209.0 ± 107.8	297 ± 159[b]	29 ± 11[c]	104 ± 24[b,c]
ABLC (whole blood)	90.5 ± 65.9[b,d]	2.4 ± 0.3	2,623 ± 2,466	3,754 ± 3,544[c]	39 ± 11[c]	19.4 ± 18.6[b]

[a] By these regimens the animals each received 1 mg of AmB per kg. The cholesterol enrichment contained 0.5% (wt/vol) cholesterol. Data are expressed as means ± standard deviations (n = 4 for Doc-AmB in plasma, n = 5 for ABLC in plasma, and n = 3 for ABLC in whole blood). $t_{1/2\alpha}$ and $t_{1/2\beta}$, α and terminal half-lives, respectively.

[b] $P < 0.05$ versus rabbits fed a regular diet.

[c] $P < 0.05$ versus Doc-AmB treatment.

[d] $P < 0.05$ versus ABLC (plasma).

TABLE 3. Distribution of AmpB in tissue following 7 consecutive days of Doc-AmB and ABLC administration to control and cholesterol-fed rabbits[a]

Tissue	AmB distribution (μg of AmB/g of tissue)			
	Control rabbits		Cholestrol-fed rabbits	
	Doc-AmB	ABLC	Doc-AmB	ABLC
Kidney	3.95 ± 1.24	0.20 ± 0.12[b]	5.43 ± 2.41	0.43 ± 0.18[b,c]
Liver	9.27 ± 2.18	8.68 ± 3.20	9.03 ± 1.82	5.12 ± 2.71
Lung	3.22 ± 2.94	1.07 ± 1.30	1.11 ± 0.68	2.77 ± 1.55
Spleen	1.31 ± 0.79	5.37 ± 3.75	3.68 ± 3.10	4.69 ± 3.57
Heart	0.11 ± 0.20	ND[d]	0.25 ± 0.06	0.02 ± 0.00[c]

[a] By these regimens the animals each received 1 mg of AmB per kg. The cholesterol enrichment containes 0.5% (wt/vol) cholesterol. Data are means ± standard deviations ($n = 4$ for Doc-AmB and $n = 5$ for ABLC).
[b] $P < 0.05$ versus Doc-AmB.
[c] $P < 0.05$ versus control rabbits.
[d] ND, below detectable limit of the assay.

olemic rabbits. This result could be explained by the fact that the systemic clearance of Doc-AmB was significantly lower in hypercholesterolemic rabbits. Furthermore, the V_{SS} of Doc-AmB was significantly lowered in hypercholesterolemic rabbits than in normolipidemic rabbits ($P < 0.05$), possibly suggesting that plasma protein and/or lipoprotein binding differences account for changes in the disposition of AmB (Fig. 3). This decrease in V_{SS} can further be explained by the increased concentrations of AmB in the livers, spleens, and kidneys of cholesterol-fed rabbits compared to those in these tissues of regular diet-fed rabbits (Table 3).

Since AmB can be associated with plasma lipoproteins, we expected a greater AUC with a reduction in CL in the presence of hypercholesterolemia. We hypothesize that this may be due to the drug's preferential association with LDLs and TRLs, the levels of which are increased in the hypercholesterolemic rabbit model used in the present study (Table 1). Consistent with this hypothesis, we observed that a greater percentage of AmB was recovered in the TRL fraction when the drug was administered to hypercholesterolemic rabbits than when it was administered to normolipidemic rabbits ($P < 0.05$) (Fig. 3A), but we observed no differences in the level of association of AmB with LDL. This may be because the increase in TRL cholesterol levels is far greater than the elevation in LDL cholesterol levels (Table 1). Taken together, these findings suggest that TRL may be an important mediator of drug disposition.

We further hypothesized that the associations of AmB with lipoproteins have major effects on the safety of this drug since Doc-AmB is often administered to patients with abnormal plasma cholesterol and triglyceride metabolism (5, 8, 12, 14, 30). Growing evidence supports our hypothesis that increases in the cholesterol concentration increase the renal toxicity of Doc-AmB (33, 34, 39), while an elevation in the plasma triglyceride concentration or an association of AmB with a triglyceride-rich emulsion (27) decreases AmB-induced renal toxicity (6). Specifically, when Doc-AmB was administered to patients with leukemia (17) and immunocompromised patients (23) who exhibited lower plasma cholesterol concentrations (<100 mg/dl), the level of AmB-induced renal toxicity was decreased. Chabot and coworkers (5) observed no measurable renal toxicity when Doc-AmB was administered to cancer patients who exhibited hypocholesterolemia. Our preliminary findings from studies with humans suggest that patients with higher serum LDL cholesterol levels and, in turn, a greater level of binding of AmB with serum LDL are more susceptible to AmB-induced kidney toxicity (33). This work was later supported by our single-dose studies with hypercholesterolemic rabbits (39).

However, in the present multiple-dose study, the increased $AUC_{0-\infty}$ for AmB in cholesterol-fed rabbits compared to that for regular diet-fed rabbits administered Doc-AmB ($P < 0.05$) (Table 2) was associated with less of an increase in plasma creatinine levels (Table 1) and no changes in the renal tissue AmB concentration (Table 3). These observations may be explained by differences in AmB's distribution in plasma lipoprotein. In cholesterol-fed rabbits, a greater percentage of AmB was recovered in the TRL fraction (which predominantly contains very-low-density lipoproteins and chylomicrons) (Fig. 3A). Although no differences in renal tissue AmB concentra-

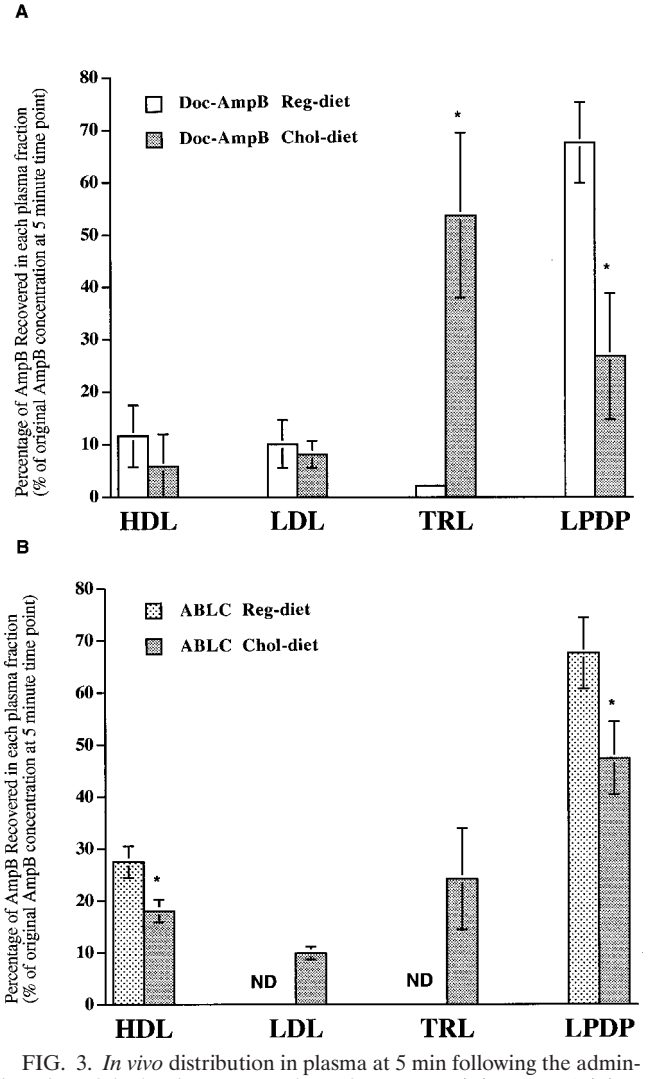

FIG. 3. *In vivo* distribution in plasma at 5 min following the administration of the last intravenous dose of Doc-AmB (A) or ABLC (B) to cholesterol (Chol)-fed or regular (Reg) diet-fed rabbits. Values are means ± standard deviations ($n = 5$), * $P < 0.05$ versus rabbits fed a regular diet and receiving Doc-AmB or ABLC. The LPDP fraction includes albumin and α-1-glycoprotein.

tions were observed following Doc-AmB administration to cholesterol-fed rabbits, AmB's increased level of association with the TRL fraction may decrease AmB's ability to inflict renal damage at the cellular level. This may be because receptor-mediated uptake of apolipoprotein B- and E-rich lipoproteins (namely, LDL and TRL) by human glomerular epithelial cells (11, 24) is downregulated in cholesterol-fed rabbits (11). Thus, most TRL-associated AmB, although delivered to the renal tissue, would not interact with kidney cells and would not cause damage.

In contrast, no significant renal toxicity was observed in either rabbit group administered ABLC (Table 1). This observation is supported by our findings that no significant differences in renal tissue AmB levels were observed (Table 3). Although renal toxicity following ABLC administration to cholesterol-fed rabbits is not significant, it is greater than that following administration of ABLC to regular diet-fed rabbits. This may be due to the increased distribution of AmB into the LDL fraction following ABLC administration to cholesterol-fed rabbits compared to that following ABLC administration to regular diet-fed rabbits (Fig. 3B). However, taken together with the lipoprotein distribution data, it appears that the increased level of association of AmB with TRL in hypercholesterolemic rabbits (Fig. 3) diminishes the AmB-induced renal toxicity.

The pharmacokinetics of ABLC and its distributions in tissue were also markedly altered in the presence of hypercholesterolemia. Whereas the transport of Doc-AmB was influenced by TRL cholesterol concentrations, preferential uptake of ABLC into the reticuloendothelial system may be a result of LDL cholesterol levels. Specifically, AmB CL following ABLC administration was significantly decreased in cholesterol-fed rabbits compared to that in regular diet-fed rabbits ($P < 0.05$). This decrease in AmB CL may be a result of ABLC's increased interaction with LDL. LDL has a circulating half-life of 24 to 48 h (34), and therefore, the interaction of ABLC with LDL could result in a longer AmB half-life and reduced systemic CL. Similar to the findings observed following Doc-AmB administration, the V_{SS} of ABLC was lower in hypercholesterolemic rabbits than in normolipidemic rabbits, possibly suggesting that plasma protein and/or lipoprotein binding differences account for changes in disposition, as reported in Fig. 3.

Furthermore, we have observed that a greater percentage of AmB associates with the LPDP fraction when ABLC was administered to these animals. An increase in cholesterol levels does alter this distribution (Fig. 3B). In contrast to the observations with Doc-AmB administration, no significant change in renal toxicity was found with ABLC dosing. These data are consistent with our previous work with rats (35) and with the work of other investigators that have demonstrated that AmB delivered in a lipid complex has a nephroprotective effect (17, 28).

Bhamra and coworkers (3) have observed similar concentration-time curves for Doc-AmB and ABLC following administration of these compounds to rats, as we did following administration of these compounds to rabbits (Fig. 2A). They further reported that when rat plasma was spiked with Doc-AmB and incubated for 3 h at 37°C, most of the drug was associated with the very-low-density liproprotein and LPDP fractions. Greater than 50% of the AmB from samples spiked with ABLC or Doc-AmB was associated with the LPDP fraction. Those findings are in agreement with our results (Fig. 3).

Preliminary studies by our laboratory (data not shown) and others (1) observed no differences in AmB's pharmacokinetics in whole blood and plasma following Doc-AmB administration. However, differences in AmB's pharmacokinetics in whole blood and plasma were observed following ABLC administration to humans (1) and rabbits (Table 2). This is due to the fact that upon separation of plasma from red blood cells by centrifugation, the ABLC-associated AmB is also partitioned into the red blood cell fraction (1). This causes an underestimation of the plasma AmpB concentration following ABLC administration, resulting in the miscalculation of the systemic pharmacokinetics of AmB from the data for plasma (1). In rabbits fed a regular diet, the AUC, the distribution and elimination half-lives, and MRT are significantly greater for AmB in whole blood than for AmB in plasma ($P < 0.05$). The CL of ABLC from whole blood is significantly lower than that from plasma ($P < 0.05$) (Table 2). Similar findings were observed in cholesterol-fed rabbits ($P < 0.05$) (Table 2). Taken together, these findings suggest that elevated plasma cholesterol levels modify the pharmacokinetics of AmB in a fashion similar to that in which they modify the pharmacokinetics of AmB in plasma (Table 2).

In conclusion, the studies described here suggest that elevations in plasma cholesterol concentrations increase the concentration of AmB recovered in apolipoprotein B- and E-rich lipoproteins (TRL and LDL). This change in the distribution of AmB in plasma results in decreased systemic CL of AmB and lower levels of AmB-induced renal toxicity in hypercholesterolemic rabbits following the administration of multiple intravenous doses of Doc-AmB or ABLC. These findings may be of importance when Doc-AmB or ABLC is administered to patients with elevated plasma cholesterol levels. However, further studies with patients are warranted.

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REFERENCES

1. **Adedoyin, A., J. F. Bernardo, C. E. Swenson, L. E. Bolsack, G. Horwith, S. DeWit, E. Kelly, J. Klasterksy, J. P. Sculier, D. DeValeriola, E. Anaissie, G. Lopez-Berestein, A. Llanos-Cuentas, A. Boyle, and R. A. Branch.** 1997. Pharmacokinetic profile of ABELCET (amphotericin B lipid complex injection): combined experience from phase I and phase II studies. Antimicrob. Agents Chemother. **41:**2201–2208.
2. **Balazsovits, J. A., L. D. Mayer, M. B. Bally, P. R. Cullis, M. McDonell, R. S. Ginsberg, and R. E. Falk.** 1989. Analysis of the effect of liposomal encapsulation on the vesicant properties, acute and cardiac toxicities, and antitumor efficacy of doxorubicin. Cancer Chemother. Pharmacol. **23:**81–86.
3. **Bhamra, R., A. Sa'ad, L. E. Bolcsak, A. S. Janoff, and C. E. Swenson.** 1997. Behaviour of amphotericin B lipid complex in plasma in vitro and in the circulation of rats. Antimicrob. Agents Chemother. **41:**886–892.
4. **Bodey, G. P.** 1986. Infection in cancer patients: a continuing association. Am. J. Med. **81:**11–26.
5. **Chabot, G. G., R. Pazdur, F. A. Valeriote, and L. H. H. Baker.** 1989. Pharmacokinetics and toxicity of continuous infusion of amphotericin B in cancer patients. J. Pharm. Sci. **78:**307–310.
6. **Chavanet, P., V. Joly, D. Rigaud, J. Bolard, C. Carbon, and P. Yenni.** 1994. Influence of diet on experimental toxicity of amphotericin B deoxycholate. Antimicrob. Agents Chemother. **38:**963–968.
7. **Davis, R. A., and J. E. Vance.** 1996. Structure, assembly and secretion of lipoproteins, pp. 473–493. *In* D. E. Vance and J. E. Vance (ed.), Biochem-

istry of lipids, lipoproteins and membranes. Elsevier, New York, N.Y.
8. **Feingold, K. R., R. M. Krauss, M. Pang, W. Doerrler, P. Jensen, and C. Grunfeld.** 1993. The hypertriglyceridemia of acquired immunodeficiency syndrome is associated with an increased prevalence of low-density lipoprotein subclass pattern. J. Clin. Endocrinol. Metab. **76:**1423–1431.
9. **Gardier, A. M., D. Mathe, X. Guedeney, J. Barre, C. Benvenutti, N. Navarro, L. Vernillet, D. Loisance, J. P. Cachera, B. Jacotot, and J. P. Tillement.** 1993. Effects of plasma lipid levels on blood distribution and pharmacokinetics of cyclosporin A. Ther. Drug Monit. **15:**274–280.
10. **Gates, C., and R. J. Pinney.** 1993. Amphotericin B and its delivery by liposomal and lipid formulations. J. Clin. Pharm. Ther. **18:**147–153.
11. **Grone, H. J., A. K. Walli, E. Grone, A. Kramer, M. R. Clemens, and D. Seidel.** 1990. Receptor mediated uptake of apo B and apo E rich lipoproteins by human glomerular epithelial cells. Kidney Int. **37:**1449–1459.
12. **Grunfeld, C., M. Pang, W. Doerrler, J. K. Shigenaga, P. Jensen, and K. R. Feingold.** 1992. Lipids, lipoproteins, triglyceride clearance and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. J. Clin. Endocrinol. Metab. **74:**2045–2051.
13. **Ha, Y. C., and P. J. Barter.** 1982. Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. Comp. Biochem. Physiol. **71:** 265–269.
14. **Kritchevsky, S. B., T. C. Wilcosky, D. L. Morris, K. N. Truong, and H. A. Tyroler.** 1991. Changes in plasma lipid and lipoprotein cholesterol and weight prior to the diagnosis of cancer. Cancer Res. **51:**3198–3203.
15. **Lopez-Berestein, G., R. Mehta, R. L. Hopfer, K. Mills, L. Kasi, K. Mehta, V. Fainstein, M. Luna, E. M. Hersh, and R. Juliano.** 1983. Treatment and prophylaxis of disseminated infection due to Candida albicans in mice with liposomal-encapsulated amphotericin B. J. Infect. Dis. **147:**939–945.
16. **Lopez-Berestein, G., M. G. Rosenblum, and R. Mehta.** 1984. Altered tissue distribution of amphotericin B by liposomal encapsulation: comparison of normal mice to mice infected with Candida albicans. Cancer Drug Deliv. **1:** 199–205.
17. **Lopez-Berestein, G.** 1988. Liposomes as carriers of antifungal drugs. Ann. N. Y. Acad. Sci. **544:**590–597.
18. **Meyer, R. D.** 1992. Current role of therapy with amphotericin B. Clin. Infect. Dis. **14:**S154–S160.
19. **Morton, R. E., and D. A. Zilversmit.** 1982. Purification and characterization of lipid transfer protein(s) from human lipoprotein-deficient plasma. J. Lipid Res. **23:**1058–1067.
20. **Norido, F., A. Zatta, C. Fiorito, M. Prosdocimi, and G. Weber.** 1993. Hematologic and biochemical analysis profiles of selectively bred WHHL rabbits. Lab. Anim. Sci. **43:**319–323.
21. **O'Meara, N. M., R. A. Devery, D. Owens, P. B. Collins, A. H. Johnson, and G. H. Tomkin.** 1991. Serum lipoproteins and cholesterol metabolism in two hypercholesterolemic rabbit models. Diabetologia **34:**139–143.
22. **Perez-Soler, R., A. R. Khokhar, M. P. Hacker, and G. Lopez-Berestein.** 1986. Toxicity and antitumor activity of *cis*-bis-cyclopentenecarboxylato-1,2-diaminocyclohexane platinum (II) encapsulated in multilamellar vesicles. Cancer Res. **46:**6269–6273.
23. **Pontaini, D. R., D. Sun, J. W. Brown, S. I. Shahied, O. J. Plescia, C. P. Schaffner, G. Lopez-Berestein, and P. S. Sarin.** 1989. Inhibition of HIV replication by liposomal encapsulated amphotericin B. Antivir. Res. **11:**119–125.
24. **Quaschning, T., M. Koniger, A. Kramer-Guth, S. Greiber, H. Pavenstadt, M. Nauck, P. Schollmeyer, and C. Wanner.** 1997. Receptor-mediated lipoprotein uptake by human glomerular cells: comparison with skin fibroblasts and HepG2 cells. Nephrol. Dial. Transplant. **12:**2528–2536.
25. **Rothon, D. A., R. G. Mathias, and M. T. Schechter.** 1994. Prevalence of HIV infection in provincial prisons in British Columbia. Can. J. Med. Assoc. **151:** S154–S160.
26. **Shargel, L., and Yu, A. B. C.** 1985. Multicompartment models, p. 51–67. *In* L. Shargel and A. B. C. Yu (ed.), Applied biopharmaceutics and pharmacokinetics. Appleton & Lange, Norwalk, Conn.
27. **Souza, L. C., R. C. Maranhao, S. Schreier, and A. Campa.** 1993. In-vitro and in-vivo studies of the decrease of amphotericin B toxicity upon association with a triglyceride-rich emulsion. J. Antimicrob. Chemother. **32:**123–132.
28. **Taylor, R. L., D. M. Williams, P. C. Craven, J. R. Graybill, D. J. Drutz, and W. E. Magee.** 1982. Amphotericin B in liposomes: a novel therapy for histoplasmosis. Am. Rev. Respir. Dis. **125:**610–611.
29. **Vadiei, K., G. Lopez-Berestein, R. Perez-Soler, and D. R. Luke.** 1991. Tissue distribution and in vivo immunosuppressive activity of liposomal cyclosporine. Drug Metab. Dispos. **19:**1147–1151.
30. **Vitols, S., G. Gahrton, M. Bjorkholm, and C. Peterson.** 1985. Hypocholesterolemia in malignancy due to elevated low-density lipoprotein receptor activity in tumor cells: evidence from studies in patients with leukemia. Lancet **ii:**1150–1154.
31. **Walsh, T. J., J. Bacher, and P. A. Pizzo.** 1988. Chronic silastic central venous catherization for reduction, maintenance and support of persistent granulocytopenia in rabbits. Lab. Anim. Sci. **38:**467–471.
32. **Wasan, K. M., M. G. Rosenblum, L. Cheung, and G. Lopez-Berestein.** 1994. Influence of lipoproteins on renal cytotoxicity and antifungal activity of amphotericin B. Antimicrob. Agents Chemother. **38:**223–227.
33. **Wasan, K. M., and J. S. Conklin.** 1997. Enhanced amphotericin B nephrotoxicity in intensive care patients with elevated levels of low-density lipoprotein cholesterol. Clin. Infect. Dis. **24:**78–80.
34. **Wasan, K. M., and S. M. Cassidy.** 1998. The role of plasma lipoproteins in modifying the biological activity of hydrophobic drugs. J. Pharm. Sci. **87:**411–424.
35. **Wasan, K. M., K. Vadiei, G. Lopez-Berestein, and D. R. Luke.** 1990. Pharmacokinetics, tissue distribution, and toxicity of free and liposomal amphotericin B in diabetic rats. J. Infect. Dis. **161:**562–566.
36. **Wasan, K. M., V. B. Grossie, Jr., and G. Lopez-Berestein.** 1994. Concentrations in serum and tissue distribution of free and liposomal amphotericin B in rats on continuous intralipid infusion. Antimicrob. Agents Chemother. **38:** 2224–2226.
37. **Wasan, K. M, R. E. Morton, M. G. Rosenblum, and G. Lopez-Berestein.** 1994. Decreased toxicity of liposomal amphotericin B is due to the association of amphotericin B with high density lipoproteins: role of lipid transfer protein. J. Pharm. Sci. **83:**1006–1010.
38. **Wasan, K. M., S. M. Cassidy, M. Ramaswamy, A. Kennedy, F. W. Strobel, S. P. Ng, and T. Y. Lee.** 1999. A comparison of step-gradient and sequential density ultracentrifugation and the use of lipoprotein deficient plasma controls in determining the plasma lipoprotein distribution of lipid-associated compound. Pharm. Res. **16:**165–169.
39. **Wasan, K. M., A. L. Kennedy, S. M. Cassidy, M. Ramaswamy, L. Holtorf, J. W. L. Chou, and P. H. Pritchard.** 1998. Pharmacokinetics, distribution in serum lipoprotein and tissues, and renal toxicities of amphotericin B and amphotericin B lipid complex in a hypercholesterolemic rabbit model: single-dose studies. Antimicrob. Agents Chemother. **42:**3146–3152.