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Short Communication

Role of Phospholipid Transfer Protein on the Plasma Distribution of Amphotericin B Following the Incubation of Different Amphotericin B Formulations

Nilesh Patankar[1] and Kishor M. Wasan[1,2]

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Purpose. The purpose of this study was to investigate the role of phospholipid transfer protein (PLTP) on the plasma distribution of amphotericin B (AmpB) following incubation with different AmpB formulations in human plasmas with varying lipid profiles.

Methods. In a first set of experiments, plasma distribution profiles of AmpB were determined following the incubation of Fungizone® and lipid-based formulations (Abelcet® and AmBisome®) at a concentration of 20 μg AmpB/mL for 5–120 min at 37°C in the plasma obtained from six different individuals (total cholesterol concentrations range between 62 and 332 mg/dL). In a second set of experiments, Abelcet®, and AmBisome® at a concentration of 20 μg AmpB/mL were incubated for 5 min at 37°C in human plasma (total cholesterol = 163 mg/dL) that had been pretreated with an antibody raised up against PLTP (1:400 v/v dilution from stock solution) for 20 min at 37°C. Following incubation, the human plasma was separated into its lipoprotein and lipoprotein-deficient fractions by density gradient ultracentrifugation and analyzed for AmpB content by high-performance liquid chromatography.

Results. The majority of AmpB was covered in the lipoprotein-deficient plasma and high-density lipoprotein (HDL) fractions following incubation of Fungizone® in human plasma. The majority of AmpB (48.7–87.2%) was recovered in the HDL fraction following incubation of Abelcet® and AmBisome® in human plasma. The presence of the PLTP antibody resulted in a 20% decrease in the percentage AmpB recovered in the HDL fraction following the incubation of Abelcet®. However, the plasma distribution of AmpB remained unchanged following the incubation of AmBisome® in plasma containing the PLTP antibody.

Conclusions. Taken together, these findings suggest indirect evidence that PLTP may play an important role in the plasma distribution profile of AmpB following the incubation of Abelcet® and may be one of the factors responsible for the preferential association of AmpB with HDL when administered as Abelcet®.

KEY WORDS: amphotericin B; lipid-based amphotericin B formulations; lipoproteins; phospholipid transfer protein.

INTRODUCTION

Amphotericin B (AmpB) is a polyene macrolide antibiotic used in the treatment of systemic fungal infections. Fungizone® is the most widely used formulation of AmpB in the treatment of several systemic fungal infections (1,2). However, its use is often limited by dose-dependent kidney toxicity, which is manifested by renal vasoconstriction with a significant decrease in the glomerular filtration rate and renal electrolyte loss (1).

Due to these adverse effects, several lipid formulations (i.e., Abelcet® and AmBisome®) have been developed with the aim of decreasing AmpB-induced nephrotoxicity while maintaining the drug's efficacy (2). AmBisome® is a liposomal preparation of AmpB prepared using hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and distearoyl phosphatidyl glycerol (DSPG). Abelcet® is a lipid complex commonly known as AmpB lipid complex (ABLC) composed of dimyristoylphosphatidyl glycerol (DMPG) and dimyristoylphosphatidyl choline (DMPC) (3).

Plasma lipoproteins are soluble macromolecular aggregates of lipid and protein, which serve to transport lipids through vascular and extravascular body fluids to cells that require them for energy and anabolic purposes (4,5). Lipoproteins have also been implicated in several other biologic processes including coagulation and tissue repair (6). Recently, our group (7) and others (8) have suggested that lipoproteins may have the additional function of modifying the biologic activity of water-insoluble drugs.

Our laboratory previously reported that AmpB-induced kidney toxicity may be due to the presence of high-affinity low-density lipoprotein (LDL) receptors expressed in the

[1] Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences, The University of British Columbia, 2146 East Mall, Vancouver, British Columbia V6T 1Z3, Canada.
[2] To whom correspondence should be addressed. (e-mail: Kwasan@interchange.ubc.ca)

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kidney, which mediate the cellular uptake of AmpB through the process of endocytosis (9). We also observed that an elevation in LDL cholesterol levels in the plasma was associated with greater AmpB-induced renal toxicity (9). In addition, we reported that AmpB was significantly less toxic to renal LLC PK1 cells when it was associated with high-density lipoproteins (HDLs) than when it was associated with LDL (10). These findings suggested that an increase in the amount of AmpB-associated LDL could result in an increase in renal cytotoxicity (10). Further studies in the transplant patients have supported this link of AmpB-induced nephrotoxicity to LDL levels in the body (11).

However, lipid formulations of AmpB, specifically Abelcet®, were found to be significantly less toxic without the loss of AmpB's antifungal activity (2,12). A number of potential mechanisms for this reduced toxicity have been reported by others (12,13). Furthermore, a number of studies were conducted in our laboratory where the distribution of AmpB was reported in various lipoprotein and nonlipoprotein fractions following the administration (*in vivo*) or incubation (*in vitro*) of Abelcet®. Results of these studies have shown that AmpB was predominantly associated with HDL. Based on this observation and observations from our renal toxicity studies (10), it was proposed that decreased renal toxicity of AmpB following Abelcet® administration may be due to its preferential association with HDL. Another important observation of this study is that elevation in LDL cholesterol levels in the plasma did not affect the distribution of drug for lipid-based formulations and resulted in the majority of the drug being recovered in the HDL fraction. Since this association with HDL may possibly be an important factor in reducing the nephrotoxicity of the drug, it is of interest to determine the potential mechanisms that can explain this preferential association.

Our group has previously reported that when AmpB was administered as a lipid complex, the majority of the AmpB associates with HDL, specifically the HDL_3 subfraction (14). We attributed this finding to the greater number of and increased protein content of HDL_3 particles as compared with HDL_2 particles. These initial findings suggested that HDL particle number and protein content could be possible reasons for the preferential association of AmpB with this lipoprotein fraction.

Further studies observed that liposomal nystatin (similar chemical structure as AmpB with the same liposomal phospholipid components) has similar plasma distribution profile as that of ABLC, with the vast majority of the drug recovered in the HDL fraction. We suggested that plasma lipoprotein distribution of liposomal nystatin may be influenced by the protein content of HDL [apolipoprotein AI (Apo AI)] (15), specifically, the DMPG–Apo AI complex based on the ability of DMPG to form a thermally stable complex with Apo AI (16). Further studies with Abelcet® demonstrated that greater than 90% of AmpB and greater than 80% of DMPG incubated in plasma were recovered in the HDL fraction (17). These results further supported the hypothesis that AmpB and DMPG may cotransfer as an intact drug lipid complex to HDL because the molar ratio of DMPG–AmpB observed within HDL is similar to the initial molar ratio within the liposome prior to incubation in human plasma.

A third possible mechanism proposed was the transfer of AmpB-associated DMPC from lipid complexes into HDL (18). From preliminary studies (unpublished results), it is observed that in case of both lipid-based formulations, the maximum amount of drug was found to be associated with HDL, irrespective of the plasma cholesterol content. A common component in these two formulations is the presence of phospholipids, suggesting that transfer of these phospholipids to HDL particles may result in the transport of AmpB to HDL as a drug lipid complex.

Phospholipid transfer protein (PLTP) (19–21) is a plasma protein primarily involved in the transfer of phospholipids from other lipoprotein fractions [primarily triglyceride-rich lipoproteins (TRLs) that are composed of chylomicrons and very low density lipoproteins] to HDL. Based on these activities of PLTP and previous results of AmpB plasma distribution following Abelcet® incubation, we hypothesize that PLTP may facilitate the preferential association of AmpB to HDL when administered as a lipid complex.

The purpose of this study was to investigate the role of PLTP on the plasma distribution of AmpB following incubation with different AmpB formulations in human plasmas with varying lipid profiles.

MATERIALS AND METHODS

Materials and Reagents

Human plasmas from fasted individual donors exhibiting varying lipid profiles were purchased from Bioreclamation Inc. (Hicksville, NY, USA). These plasma samples were pretested by a Food and Drug Administration (FDA)-approved test and found negative for human T-cell lymphotropic viruses (HTLVs), hepatitis B surface antigen (HBsAG), syphilis, human immunodeficiency virus (HIV 1/2), HIV-1 AG, and hepatitis C virus (HCV). Fungizone®, Abelcet®, and AmBisome® were purchased from the Department of Pharmacy at Vancouver General Hospital (Vancouver, BC, Canada). Sodium bromide was purchased from Sigma Company (St. Louis, MO, USA). PLTP Activity Kit was purchased from Cardiovascular Targets, Inc. (New York, USA). Polyclonal antibodies raised up against PLTP were purchased from Novus Biologicals, Inc. (Littleton, CO, USA).

Plasma Lipoprotein Separation

Plasma was separated into its different lipoproteins (i.e., HDL, LDL, TRL) and lipoprotein-deficient plasma (LPDP) fractions using density gradient ultracentrifugation as previously described (14,15).

To assure that AmpB distribution is not due to the density of the AmpB formulations, Fungizone®, Abelcet®, and AmBisome® were incubated in sodium bromide solutions that did not contain human plasma. Sample preparation was carried out using the same procedure as described above, and fractions separated were analyzed for AmpB content. It was observed that the majority of the drug (>92%) was recovered in the density fraction from 1.21 to 1.25 g/mL,

Table I. Total Cholesterol (TC) and Total Triglyceride (TG) Concentrations of Human Plasmas from Six Different Individuals

Plasma sample	Human plasma concentrations (mg/dL)	
	TC	TG
I	332	583
II	217	302
III	186	127
IV	178	147
V	176	122
VI	62	58

suggesting that the distribution of AmpB is not a function of formulation density (data not shown).

Measurement of AmpB from Lipoprotein Fractions

AmpB was quantified in each lipoprotein and LPDP fraction using a liquid–liquid extraction method previously described (14). AmpB concentrations were quantified against external calibration curves using a concentration range of 0.625–10 μg/mL (14). The AmpB HPLC assay has an intraday coefficient of variation (CV) of 5% and is linear in the range of 50–5000 ng/mL (14).

Experimental Design

Time Course Studies

Plasma distribution profiles of AmpB were determined following the incubation of Fungizone® and lipid-based formulations (Abelcet® and AmBisome®; AmpB concentration of 20 μg AmpB/mL) for 5–120 min at 37°C in nonfasted plasmas from six different individuals [Table I; total cholesterol (TC) concentrations range between 62 and 332 mg/dL; total triglyceride concentrations range between 58 and 583 mg/dL]. Following incubation, the plasma was cooled down to 4°C (to prevent any drug redistribution) and separated into its lipoprotein and lipoprotein-deficient fractions by density gradient ultracentrifugation. Each fraction was assayed for AmpB by HPLC.

Effect of Decreased PLTP Activity on the Plasma Distribution of AmpB Following Incubation of Abelcet® and AmBisome® in a Representative Human Plasma

Decreased PLTP Activity. The purpose of this study was to determine the optimum dilution (concentration) of the PLTP antibody from the stock solution that can be used to obtain decreased phospholipid transfer activity. A series of antibody dilutions from the stock solution provided were prepared in the same buffer [10 mM Tris, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA)] that is used for assaying PLTP activity. PLTP activity assay was carried out using these antibody dilutions in the same set of plasma (TC = 163 mg/dL) that was used for characterizing the PLTP activity. Plasma in the absence of antibody was used as a positive control. Antibody dilutions used for the experiments were 1:100 v/v, 1:400 v/v, 1:600 v/v, and 1:1000 v/v from the antibody stock solution (22,23).

AmpB Plasma Distribution. Abelcet® and AmBisome® at an AmpB concentration of 20 μg AmpB/mL were incubated for 5 min at 37°C in a fasted human plasma (note that in fasted human plasma, VLDL is the predominant TRL fraction and, thus, only VLDL distribution was determined; TC = 163 mg/dL) that had been pretreated with PLTP polyclonal antibody (1:400 v/v dilution as determined above) for 20 min at 37°C. Following incubation, the human plasma

Table II. Plasma Distribution of AmpB Following the Incubation of Fungizone®, Abelcet®, and AmBisome® in Human Plasmas from Six Different Individuals for 5, 30, 60, and 120 min at 37°C

Treatment group (% incubated)	Incubation time (min)	TRL (% recovery)	LDL (% recovery)	HDL (% recovery)	LPDP (% recovery)	Total recovery (%)
Fungizone®	5	2.3 ± 0.6	11.5 ± 4.3	20.7 ± 3.9	62.9 ± 14.9	97.4 ± 11.7
	30	2.5 ± 0.5	2.5 ± 1.5[a]	42.3 ± 2.0[a]	42.8 ± 7.2	90.1 ± 5.6
	60	7.8 ± 2.0[a]	5.7 ± 4.0	26.0 ± 5.2	40.3 ± 8.7	79.9 ± 11.6
	120	5.8 ± 0.5[a]	12.0 ± 3.8	29.5 ± 9.5	37.5 ± 6.5[a]	84.8 ± 9.6
Abelcet®	5	ND	ND	76.7 ± 10.7[b]	19.7 ± 3.7[b]	96.4 ± 8.7
	30	ND	ND	48.7 ± 4.8[a,b]	31.0 ± 6.3	79.7 ± 2.9
	60	ND	ND	64.7 ± 4.9[b]	19.5 ± 2.4[b]	84.2 ± 8.9
	120	ND	ND	46.5 ± 4.2[a,b]	34.8 ± 4.5	81.4 ± 4.7
AmBisome®	5	ND	1.7 ± 0.7[b]	71.5 ± 2.8[b]	7.5 ± 1.3[b,c]	80.7 ± 2.3
	30	ND	2.7 ± 1.7	68.7 ± 4.8[b,c]	6.0 ± 2.0[b,c]	77.4 ± 3.6
	60	0.2 ± 0.3[b]	1.8 ± 0.3	84.2 ± 2.0[a,b,c]	6.3 ± 1.5[b,c]	92.5 ± 1.8
	120	ND	2.8 ± 0.5[b]	87.2 ± 3.3[a,b,c]	8.3 ± 0.7[b,c]	98.3 ± 3.3

Data presented as mean ± standard deviation (n = 6; plasma samples from six different individuals as listed in Table I).
AmpB, amphotericin B; TRL, triglyceride-rich lipoprotein fraction (which includes chylomicrons and very low density lipoproteins); LDL, low-density lipoprotein fraction; HDL, high-density lipoprotein fraction; LPDP, lipoprotein-deficient lipoprotein fraction (which includes albumin and alpha-1-glycoprotein); ND, nondetectable, below the detectable limit of the HPLC assay.
[a] $p < 0.05$ *vs*. 5 min.
[b] $p < 0.05$ *vs*. Fungizone®.
[c] $p < 0.05$ *vs*. Abelcet®.

Table III. Rate of PLTP-Mediated Phospholipid Transfer (in picomoles per milliliter per minute) within Human Plasma in the Absence (Control) and Presence of Increasing Concentrations of a Polyclonal Antibody Raised Up Against PLTP

Antibody dilution (concentration)	Incubation time (min)				
	0 to 5	5 to 10	10 to 15	15 to 20	20 to 25
Control (no antibody)	ND	0.50 ± 0.02	2.6 ± 0.1	6.0 ± 0.5	11.1 ± 1.1
1:100 v/v	ND	ND	0.8 ± 0.02*	3.2 ± 0.2*	5.7 ± 0.6*
1:400 v/v	ND	ND	0.9 ± 0.01*	3.6 ± 0.2*	5.2 ± 0.4*
1:600 v/v	ND	ND	2.2 ± 0.03	4.6 ± 0.2	5.5 ± 0.2*
1:1000 v/v	ND	ND	1.7 ± 0.2	4.6 ± 0.3	6.0 ± 0.3*

Data presented as mean ± standard deviation (n = 6; human plasma total cholesterol = 163 mg/dL; total triglyceride = 100 mg/dL); ND, nondetectable phospholipid transfer rate.
*$p < 0.05$ *vs.* control (no antibody).

was separated into its lipoprotein and lipoprotein-deficient fractions and AmpB content was analyzed as previously described (14). The incubation time for the polyclonal antibody was based on our phospholipid transfer results reported in Table III, where a 40–50% decrease in phospholipid transfer rate was observed, as compared with untreated controls.

Statistical Analysis

The plasma distribution of AmpB was compared among different AmpB formulations and was analyzed by applying analysis of variance (ANOVA). Significant differences were assessed using Student-Newman-Keuls *post hoc* test.

A Student's t test was applied to determine the difference between the presence and absence of antibody during PLTP study. Results were considered significant if the probability of the result that occurred by chance is less than 5% ($p < 0.05$). All data were expressed as mean ± SD of $n = 6$ unless stated otherwise.

RESULTS AND DISCUSSION

Table II presents the plasma distribution of AmpB following the incubation of Fungizone®, Abelcet®, and AmBisome® at different incubation times. The majority of AmpB was covered in the LPDP and HDL fractions following incubation of Fungizone® in human plasma. However, the majority of AmpB (48.7–87.2%) was recovered in the HDL fraction following incubation of Abelcet® and AmBisome® in human plasma. According to our hypothesis, this preferential HDL association in case of lipid-based formulations may be due to phospholipid transfer activity of PLTP. As PLTP is involved in the transfer of phospholipids from TRL (VLDL, chylomicrons) to HDL, it is possible that it may transport drug complexed with the phospholipids from lipid particles to HDL. To test this hypothesis, we have assayed the phospholipid transfer activity of PLTP in the next set of experiments.

Table III presents the phospholipid transfer observed over a period of 0–25 min (in 5-min intervals) using various dilutions of antibody. After comparing with the untreated control, a statistically significant decrease in the phospholipid transfer rate was observed at an antibody dilution of 1:400 v/v 10–25 min following incubation. Based on these results, it was

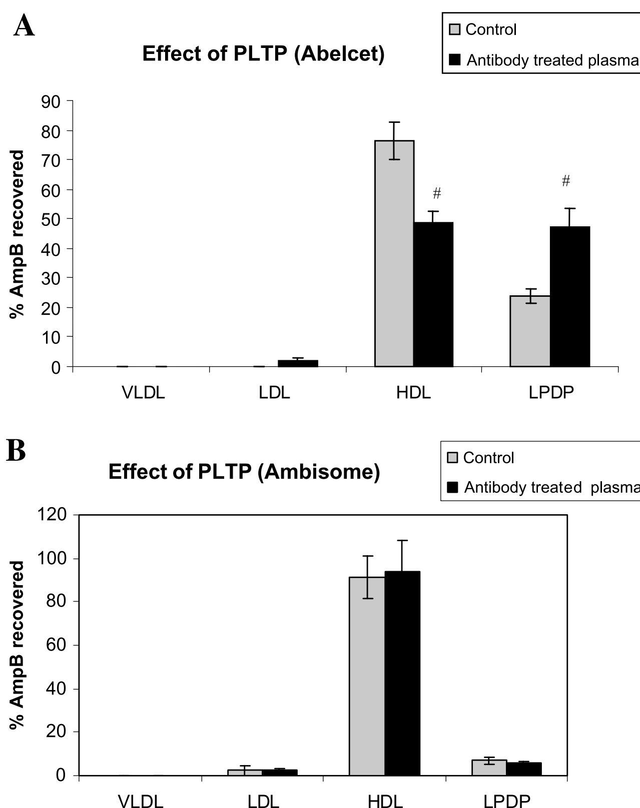

Fig. 1. (A) Effect of PLTP antibody treatment on the plasma distribution of AmpB following the incubation of Abelcet® for 5 min at 37°C in fasted human plasma (total cholesterol = 163 mg/dL) pretreated or not pretreated (control) with a PLTP polyclonal antibody (antibody dilution concentration 1:400 v/v). Values are mean ± SD, $n = 6$; #$p < 0.05$ *vs.* control. Note that in fasted human plasma, VLDL is the predominant triglyceride-rich lipoprotein fraction and, thus, only VLDL distribution was determined. (B) Effect of PLTP antibody treatment on the plasma distribution of AmpB following the incubation of AmBisome® for 5 min at 37°C in fasted human plasma (total cholesterol = 163 mg/dL) pretreated or not pretreated (control) with a PLTP polyclonal antibody (antibody dilution concentration 1:400 v/v). Values are mean ± SD, $n = 6$. Note that in fasted human plasma, VLDL is the predominant triglyceride-rich lipoprotein fraction and, thus, only VLDL distribution was determined.

decided that 1:400 v/v antibody dilution and 20 min incubation time would be used for the next set of experiments.

Figure 1 presents the plasma distribution of AmpB following the incubation of Abelcet® and AmBisome®, respectively, in control and plasma treated with PLTP antibody. The presence of the PLTP antibody resulted in a 20% decrease in the percentage AmpB recovered in the HDL fraction following the incubation of Abelcet® (Fig. 1A); however, the plasma distribution of AmpB remained unchanged following the incubation of AmBisome® in plasma treated with the PLTP antibody (Fig. 1B). Our findings following the incubation of Abelcet® provides indirect evidence that PLTP may play a role in the plasma distribution of AmpB when it is administered as a lipid complex and, therefore, could be one of the factors responsible for preferential association of AmpB with HDL. However, the presence of the PLTP antibody clearly had no effect of AmpB plasma distribution following the incubation of AmBisome®. A possible explanation for the different results reported for Abelcet® and AmBisome® could be attributed to the lipid constituents that comprise these formulations. Abelcet® is composed of DMPC–DMPG (7:3 molar ratio) as carrier phospholipids. Thus, when phospholipids transfer activity is decreased, the transfer of drug complexed with these phospholipids to HDL decreases. However, AmBisome® is composed of HSPC–DSPG–cholesterol(2:1:0.8 molar ratio). Since AmpB has a high affinity for sterol components (24), we hypothesize that AmpB's affinity for cholesterol present in this formulation prevents the ability of PLTP to transfer the drug to HDL. Therefore, inhibition of PLTP activity does not have an effect on the transfer of AmpB into the HDL fraction. These findings further suggest that AmpB's distribution into HDL following the incubation of AmBisome® may be due another unknown mechanism that warrants further investigation.

In conclusion, these findings suggest indirect evidence that PLTP may play an important role in the plasma distribution profile of AmpB following the incubation of Abelcet® and may be one of the factors responsible for the preferential association of AmpB with HDL when administered as Abelcet®.

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REFERENCES

1. R. Sabra and R. A. Branch. Amphotericin B nephrotoxicity. *Drug Safety* **5**:94–108 (1990).
2. S. Shoham and T. J. Walsh. Lipid formulations of amphotericin B: current roles in management of invasive fungal infections. *Curr. Treat. Options Infect. Dis.* **4**:535–547 (2002).
3. G. Storm and D. J. A. Crommelin. Liposomes: quo vadis? *PSTT* **1**:19–31 (1998).
4. J. Babiak and L. L. Rudel. Lipoproteins and atherosclerosis. *Baillieres Clin. Endocrinol. Metab.* **1**:515–550 (1987).
5. R. A. Davis, and J. E. Vance. Structure, assembly and secretion of lipoproteins. In D. E. Vance and J. E. Vance (eds.), *Biochemistry of Lipids, Lipoproteins and Membranes*, Elsevier, New York, NY, 1996, pp. 473–493.
6. A. Mbewu and P. N. Durrington. Lipoprotein (a): structure, properties and possible involvement in thrombogenesis and atherogenesis. *Atherosclerosis* **85**:1–14 (1990).
7. K. M. Wasan and S. M. Cassidy. Role of plasma lipoproteins in modifying the biological activity of hydrophobic drugs. *J. Pharm. Sci.* **87**:411–424 (1998).
8. E. G. Arathoon. Clinical efficacy of echinocandin antifungals. *Curr. Opin. Infect. Dis.* **14**:685–691 (2001).
9. M. Ramaswamy, K. Peteherych, A. Kennedy, and K. Wasan. 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. *Antimicrob. Agents Chemother* **45**:1184–1191 (2001).
10. K. M. Wasan, M. G. Rosenblum, L. Cheung, and G. Lopez-Berestein. Influence of lipoproteins on renal cytotoxicity and antifungal activity of amphotericin B. *Antimicrob. Agents Chemother.* **38**:223–227 (1994).
11. K. M. Wasan and J. S. Conklin. Enhanced amphotericin B nephrotoxicity in intensive care patients with elevated levels of low-density lipoprotein cholesterol. *Clin. Infect. Dis.* **24**:78–80 (1997).
12. J. P. Barrett, R. A. Vardulaki, C. Conlon, J. Cooke, P. Daza-Ramirez, and E. G. Evans *et al*. A systematic review of the antifungal effectiveness and tolerability of amphotericin B formulations. *Clin. Ther.* **5**:1295–1320 (2003).
13. S. Costa and N. Nucci. Can we decrease amphotericin nephrotoxicity? *Curr. Opin. Crit. Care* **7**:379–383 (2001).
14. A. Kennedy and K. Wasan. Preferential distribution of amphotericin B lipid complex into human HDL_3 is a consequence of high density lipoprotein coat lipid content. *J. Pharm. Sci.* **88**:1149–1155 (1999).
15. S. M. Cassidy, F. W. Strobel, and K. M. Wasan. Plasma lipoprotein distribution of liposomal nystatin is influenced by protein content of high density lipoproteins. *Antimicrob. Agents Chemother.* **42**:1878–1888 (1998).
16. W. K. Surewicz, R. M. Epand, H. J. Pownall, and S. W. Hui. Human apolipoprotein A-I forms thermally stable complexes with anionic but not with zwitterionic phospholipids. *J. Biol. Chem.* **261**:16191–16197 (1986).
17. K. M. Wasan, G. A. Brazeau, A. Keyhani, A. C. Hayman, and G. Lopez-Berestein. Roles of liposome composition and temperature in distribution of amphotericin B in serum lipoproteins. *Antimicrob. Agents Chemother.* **37**:246–250 (1993).
18. K. M. Wasan and R. Perez-Soler. Distribution of free and liposomal annamycin with human plasma is regulated by plasma triglyceride concentrations but not by lipid transfer protein. *J. Pharm. Sci.* **84**:1094–1100 (1995).
19. A. R. Tall, L. R. Forester, and G. L. Bongiovanni. Facilitation of phosphatidylcholine transfer into HDL lipoproteins by an apolipoprotein in the density 1.20–1.26 g/ml fraction of plasma. *J. Lipid Res.* **24**:277–289 (1983).
20. J. J. Albers, J. H. Tollefson, C. H. Chen, and A. Steinmetz. Isolation and characterization of human plasma lipid transfer proteins. *Arteriosclerosis* **4**:49–58 (1984).
21. H. L. Nishida and T. Nishida. Phospholipid transfer protein mediates transfer of not only phosphatidylcholine but also cholesterol from phosphatidylcholine-cholesterol vesicles to high density lipoproteins. *J. Biol. Chem.* **272**:6959–6964 (1997).
22. S. J. Murdoch, G. Wolfbauer, H. Kennedy, S. M. Marcovina, M. C. Carr, and J. J. Albers. Differences in reactivity of antibodies to active *versus* inactive PLTP significantly impacts PLTP measurement. *J. Lipid Res.* **43**:281–289 (2002).
23. J. B. Massey, D. Hickson-Bick, D. P. Via, A. M. Gotto, and H. J. Pownall. Fluorescence assay of the specificity of human plasma and bovine liver phospholipids transfer proteins. *Biochim. Biophys. Acta* **835**:124–131 (1985).
24. J. Adler-Moore and R. T. Proffitt. Ambisome®: liposomal formulation, structure, mechanism of action and pre-clinical experience. *J. Antimicrob. Chemother.* **49**:21–30 (2002).