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ORIGINAL ARTICLE

Differences in human phospholipid transfer protein activity following incubation of Fungizone® compared to lipid-based Amphotericin-B formulations in normolipidemic and hyperlipidemic plasma

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Abstract

Aim: To investigate how different formulations of Amphotericin-B (Amp-B) affect the activity of phospholipid transfer protein (PLTP) when incubated with hyperlipidemic and normolipidemic plasma at physiological temperature (37°C). *Methods*: Six hyperlipidemic and six normolipidemic plasma samples were collected and tested for protein concentration. Equivalent protein levels (25 μg) were then tested for PLTP activity using an in vitro established kit at physiological temperature (37°C). Increasing concentrations of different Amp-B formulations (1, 2, and 5 μg/mL) in the pharmacological range were then added to the plasma and tested for activity from 5 to 90 minutes. The Amp-B formulations used in the study were Fungizone®, Abelcet®, and AmBisome®. *Results*: In normolipidemic plasma, PLTP activity was found to be increased by Abelcet® and AmBisome® but inhibited by Fungizone®. In hyperlipidemic plasma, PLTP activity was found to be increased by Abelcet® and AmBisome® but not changed by Fungizone®. The Vm value for Abelcet® and AmBisome® was higher than Fungizone®; although, no difference was observed in the Km values between formulations. *Conclusions*: Findings suggest that lipid-based formulations of Amp-B promote the transfer of Amp-B into high-density lipoprotein fractions at a degree of increase inversely proportional to the lipid levels in the plasma.

Key words: *Abelcet®; AmBisome®; amphotericin-B; Fungizone®; phospholipid transfer protein*

Introduction

Plasma phospholipid transfer protein (PLTP) is a protein that in plasma transfers phospholipids from triglyceride-rich lipoproteins such as very low-density lipoproteins and low-density lipoproteins (VLDL/LDL) to high-density lipoproteins (HDL). It also functions in the remodeling of HDL of which along with cholesteryl ester transfer protein (CETP) aid in the reverse cholesterol transport pathway[1–4].

The molecular model for PLTP shows two barrel domains that contain hydrophobic pockets: the N-terminal pocket being responsible for PLTP activity and the C-terminal pocket being responsible for HDL remodeling (Figure 1). The PLTP molecule while still largely not well understood because of not having a defined crystallization structure, is known to contain many O- and N-type glycosylation sites that naturally increase its molecular weight in plasma over the recombinant molecular weight size of 72.64 kDa[1,5].

PLTP is distributed as a combination of high-activity PLTP (HA-PLTP) and low-activity PLTP (LA-PLTP) in human plasma[4]. HA-PLTP is associated with the majority of all PLTP activity and its functioning and has been found to be associated with Apolipoprotein-A1 (through apolipoprotein adsorption studies)[6]. The binding of Apolipoprotein-A1 with PLTP is widely seen as the point of transfer for lipolytic surface remnants into pre-β-HDL

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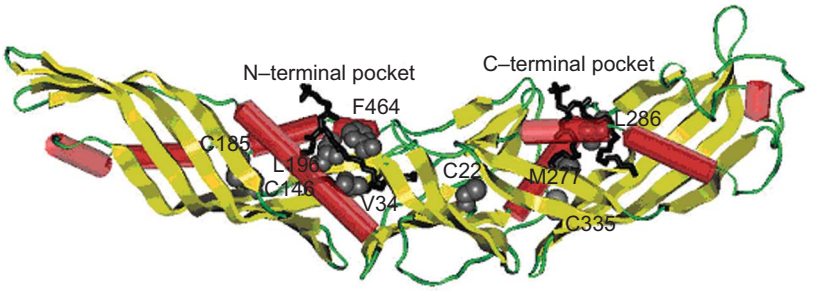

Figure 1. Molecular model of PLTP showing the molecules ability for N- and O-type glycosylation and both the N-terminal and C-terminal pocket of which are responsible for PLTPs main actions[5].

and signifies HA-PLTPs classification change into LA-PLTP. Currently not much is known about the functioning of LA-PLTP, but it is known that it has a much higher molecular weight because of extra binding associations and that it can become activated to a greater extent through binding to Apolipoprotein-E[7].

This basic model for PLTP suggests a mechanism that is antiatherogenic in action with PLTP transferring lipolytic surface remnants into pre-β-HDL[1,8,9]. However, research has revealed that while the basic nature of PLTP is antiatherogenic its mechanisms are also associated with inflammatory markers and especially an increase in the expression of Apolipoprotein-B (Apo-B) of which are key proatherogenic markers[1,10–13]. This atherogenic nature of PLTP is attributed only to plasma PLTP at present with macrophage-derived PLTP typically being considered antiatherogenic because of it corresponding to Apo-E release[9,14,15]. It is also possible that the distribution profile of HA-PLTP to LA-PLTP plays just as big a role in overall PLTP effects in the body as changing activity levels from HA-PLTP, but for this to be shown more work on revealing LA-PLTP functioning is needed[16].

CETP is a molecule similar to PLTP; CETP is predominantly bound to HDL and redistributes cholesteryl esters from HDL to triglyceride-rich lipoproteins and triglycerides from triglyceride-rich lipoproteins to LDL and HDL[17]. It has been found that CETP activity is inhibited by lipid formulations of Amphotericin-B (Amp-B)[17].

Amp-B is a polyene macrolide antibiotic drug most often used in the hospital setting to treat systemic fungal infections[18]. Fungizone® is a micellar Amp-B dispersion that is distributed in lipid-deficient plasma fractions. However, Fungizone® is associated with a high incidence of dose-dependent nephrotoxicity because of its distribution profile in the body[18]. Thus, other formulations of Amp-B (lipid-based) have been developed so as to limit the nephrotoxicity of Amp-B by changing its distribution profile and thus allowing for greater concentration of drug to be delivered to patients.

Abelcet® is one such Amp-B formulation and is a lipid-complex of dimyristoylphosphatidyl choline and dimyristoylphosphatidyl glycerol (DMPC and DMPG in a 7:3 molar ratio) that becomes bound to HDL and has been shown to inhibit cholesteryl ester and Amp-B transfer in a unidirectional manner from HDL to LDL as was found in a previous study in our laboratory[18].

AmBisome® another Amp-B formulation is also available, consisting of a lipid-complex that uses hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and distearoyl phosphatidyl glycerol (HSPC, DSPG, and cholesterol in a 2:1:0.8 molar ratio)[18].

It has previously been shown that Amp-B is taken up into PLTP and distributed on a fractional basis to HDL of which is the location of HA-PLTP and essentially the majority of PLTP's measurable activity[18]. Thus, it was hypothesized that the action of Amp-B uptake into PLTP may reduce the activity of PLTP because of increased binding.

In a recent study by our laboratory, Patankar et al.[18] found that PLTP may play a role in the distribution profile of Amp-B following the incubation of Abelcet® and in particular distribute Amp-B to HDL fractions of plasma. It was therefore hypothesized that as HA-PLTP is seen to correspond with HDL fractions in its mechanism that Amp-B formulations are being taken up by the HA-PLTP fraction of PLTP. If the lipid formulation of Amp-B aided in transportation of Amp-B into HDL fractions in the plasma then this could be a significant reason why liposomal forms of Amp-B are less nephrotoxic.

Materials and methods

Chemicals and plasma

Abelcet® (ABLC; Enzon Pharmaceuticals Inc., Piscataway, NJ, USA), AmBisome®, and Fungizone® (Bristol Myers Squibb, Nutley, NJ, USA) were purchased from the Vancouver General Hospital Department of Pharmacy Services (Vancouver, BC, Canada). Human plasma samples were obtained after quantification of cholesterol and

triglyceride levels from Bioreclamation (East Meadow, NY, USA). PLTP Activity Assay Kits were obtained from Roar Biomedical (New York, NY, USA). Recombinant PLTP was purchased from Abnova Corporation (Niehu, Taipei, Taiwan).

Plasma samples

Protein levels quantification

Twelve different human plasma samples were obtained from donors representing both normolipidemic plasma (n = 6) and hyperlipidemic plasma (n = 6) (Table 1) based on the standards set by the Ministry of Health and Welfare of Japan (cholesterol <220 mg/dL and triglycerides <150 mg/dL)[19]. This measure of hyperlipidemic plasma was used over North American standards primarily because the Japanese model uses the lower triglyceride levels. This is because higher triglyceride levels have been shown to correlate with PLTP distribution disturbance in human plasma between HA and LA PLTP and could thus cause greater initial variability in plasma sample PLTP activity. Indeed, in North American standards a triglyceride level of between 150 and 200 mg/dL is considered to be elevated, although still in the normolipidemic range.

Abelcet® and AmBisome®

Abelcet®: Ribbon-like complex with diameter between 1 and 10 μm and of a fluid phospholipid nature[20].

AmBisome®: Unilamellar liposome with diameter less than 0.1 μm and of a rigid phospholipid cholesterol nature[20].

HDL and LDL Level Quantification

Plasma samples were tested for HDL using a standardized assay and LDL levels were then quantified using the equation: LDL C = Total C – [HDL C + (Total TG)/5].

Table 1. Triglyceride and cholesterol levels of 12 plasma samples used in the study.

	Normolipidemic plasma sample		Hyperlipidemic plasma sample	
Plasma sample	Total cholesterol (mg/dL)	Total triglycerides (mg/dL)	Total cholesterol (mg/dL)	Total triglycerides (mg/dL)
1	142	142	264	269
2	101	54	391	945
3	155	107	225	204
4	117	77	237	216
5	111	30	242	240
6	166	61	267	691

Western blot analysis and densitometry and PLTP quantification

A series of western blots using 7% polyacrylamide gels were carried out in which a primary PLTP antibody quantified recombinant PLTP of known concentrations alongside plasma samples of known protein concentration. Thus, an indirect measure of protein concentration to PLTP concentration in plasma was established.

PLTP Assay

Each plasma sample was tested for PLTP activity using an activity assay that measures in vitro phospholipid transfer activity. Initially, the PLTP activity test was studied for optimum loading parameters of plasma protein and was decided to be 25 μg. The PLTP activity kit was used at a set protein concentration in plasma (25 μg) and three pharmacological concentrations of Amp-B drug formulations (Fungizone®, Abelcet®, and AmBisome®) at pharmacological levels of 1, 2, and 5 μg/mL[21]. Controls were added in the form of a picomole standard to quantify PLTP florescence levels, a blank control with only buffer, and a control with drug only. Measurements were taken for six different plasma samples in the hyperlipidemic range and six different plasma samples in the normolipidemic range. These samples were done in duplicate on each 96-well test plate, and each test plate was repeated at least twice for each plasma sample.

Statistical analysis

The groups tested in this study were compared against each other by applying a repeated measure analysis of variance (ANOVA) test and blocking results in set plasmas to account for base PLTP activity variance. Statistical differences in the data were considered significant only if the P value found was <0.05. Data added for each plasma measure consisted of an n = 6 of which each n itself had at least a replicate value of 2 done in two different plates of which itself used at least two measurements per plate to limit testing error in samples.

Results

The study began with the quantification of the protein levels in 12 different human plasma samples [hyperlipidemic (n = 6) and normolipidemic (n = 6)]. These protein levels were quantified using a BSA standard as a control of which allowed for plasma protein levels to be controlled for in future tests via adjusting plasma volume additions.

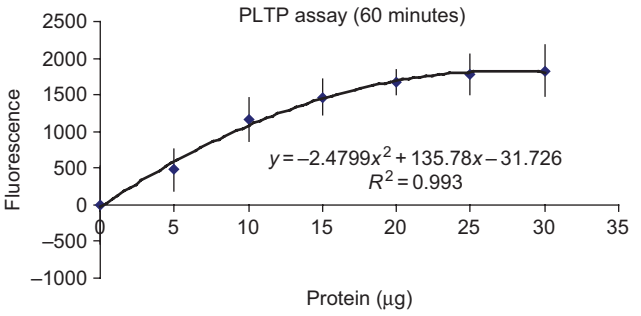

Figure 2. Plasma samples were tested for activity at different protein levels ranging from 0 to 50 µg protein to determine maximal PLTP activity levels. These tests resulted in a 0- to 30-µg protein range increasing in 5-µg increments in which the 25-µg protein level was determined as having maximal PLTP activity at physiological temperature. It has been shown in tests that elevation of temperature will increase activity but not change activity pattern.

After finding the protein concentration levels in the 12 plasma samples, assays were run to determine an appropriate amount of protein that would achieve maximal PLTP activity. This amount was measured at the incubation time points of 30, 60, and 90 minutes with the 60-minute time point showing maximal effect. At this time point it can be determined that the plasma protein concentration of approximately 25 µg protein produces a maximal amount of PLTP activity; as seen in (Figure 2).

Additionally, work was done to further quantify the plasma samples themselves by analyzing the PLTP activity of each plasma sample at set time points of incubation. The HDL and LDL of each plasma sample was measured either by quantification by Bioreclamation or by using a standardized HDL assay and quantifying for LDL using the equation: LDL C = Total C – [HDL C+(Total TG)/5]. Table 2 demonstrates the net result of these measurements.

Plasma samples analysis

PLTP western blots were then done on various plasma samples so as to compare PLTP concentrations in each plasma sample with set amount of plasma protein loaded (40 µg) (Figure 3 and Table 3). This was done with the addition of looking at recombinant PLTP at known concentrations of 400, 800, and 1200 ng. The recombinant PLTP densitometry results were compared with PLTP densitometry results with plasma samples using the same polyacrylamide gel. The recombinant PLTP on western blot analysis displayed a size consistent with its literature reported value of 72.65 kDa. However, the resulting molecular weights of the PLTP in the plasma samples were substantially higher (130–170 kDa

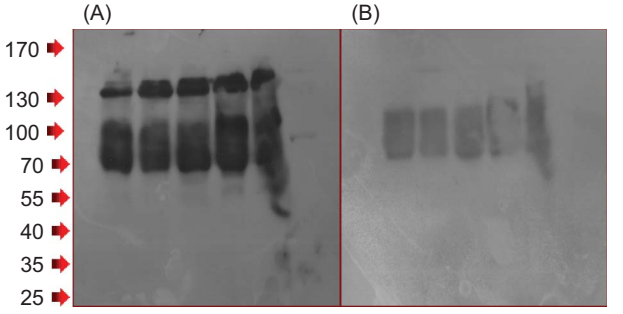

Figure 3. (A) Western blot showing PLTP bands with background noise via the addition of primary and secondary antibodies. Each lane represents a different plasma sample loaded with 40 µg of plasma protein. (B) Western blot to confirm that band seen in Figure 5A were indeed PLTP. This western blot was used to find just the background noise by only using secondary antibody.

Table 3. PLTP concentration in each plasma sample (containing 25 µg/mL protein) from western blot data that quantified recombinant PLTP bands and increasing concentrations of PLTP of 400, 800, and 1200 ng. This western blot contained a control plasma band for PLTP so as to compare different western blots.

Plasma sample	Normolipidemic plasma PLTP (ng)	Hyperlipidemic plasma PLTP (ng)
1	0.83	0.27
2	0.91	0.55
3	1.08	0.90
4	1.13	1.07
5	1.15	1.26
6	1.33	0.91

Table 2. PLTP activity analysis of 12 plasma samples using the same numeral schematic as in Table 1.

Plasma sample	Normolipidemic plasma sample			Hyperlipidemic plasma sample		
	PLTP activity (60 minutes) (pmol)	HDL (mg/dL)	Calculated LDL (mg/dL)	PLTP activity (60 minutes) (pmol)	HDL (mg/dL)	Calculated LDL (mg/dL)
1	38.82	28	85	12.61	88	122
2	42.58	49	41	25.50	82	121
3	50.35	23	110	41.87	32	152
4	52.52	26	76	49.82	46	148
5	53.85	50	55	58.63	55	139
6	62.07	39	114	42.58	36	87

as opposed to 72.64 kDa from the recombinant PLTP) than were seen in the recombinant PLTP. This increase was predicted with the many O- and N-type glycosylations that occur in plasma and is supported by other literature reports[1]. The PLTP mass also corresponded with the reported size of HA-PLTP and not the LA-PLTP fraction. Because HA-PLTP is responsible for substantially all PLTP activity, the level of PLTP in each plasma sample loaded can be quantified to an approximate level for all plasma samples. Although, when testing net activity in plasma groups, it was decided to use protein levels of plasma instead of relying on PLTP level approximation due to protein levels being more accurately quantified and due to this approach not limiting potential secondary reactions with other proteins in the system that are known to play a significant role in PLTP activity.

Once all the plasma samples had been properly quantified, assay tests on the samples' PLTP activity with the addition of Amp-B formulations (Fungizone®, Abelcet®, and AmBisome®) were carried out. Amp-B additions at pharmacological concentrations of 1, 2, and 5 µg/mL to the plasma samples when put into PLTP activity assay kit revealed statistically significant differences. These differences occurred over time as PLTP was incubated for 90 minutes at physiological temperature of 37°C. The most profound changes occurred to PLTP as both drug concentrations and incubation time increased.

Figures 4A–5B illustrate the results of these Amp-B additions to plasma samples ($n = 6$) in both hyperlipidemic and normolipidemic plasma. The activity rate of change data is calculated by subtracting basal activity at start of incubation from activity at a given incubation time point. It shows that in normolipidemic and hyperlipidemic plasma both the Abelcet® and the AmBisome® have increased PLTP activity and that they have similar effects. It should be noted that Abelcet® and AmBisome® appeared to have much more similar activity profiles in hyperlipidemic than normolipidemic plasma in which there were cases of statistical difference with other groups for one of the lipid formulations but not from the other. As far as Fungizone® is concerned, it had a very different profile from both Abelcet® and AmBisome® in normolipidemic and hyperlipidemic plasma. Fungizone® addition showed a decrease in activity from the plasma control in normolipidemic plasma but showed no change in activity from plasma with hyperlipidemic plasma.

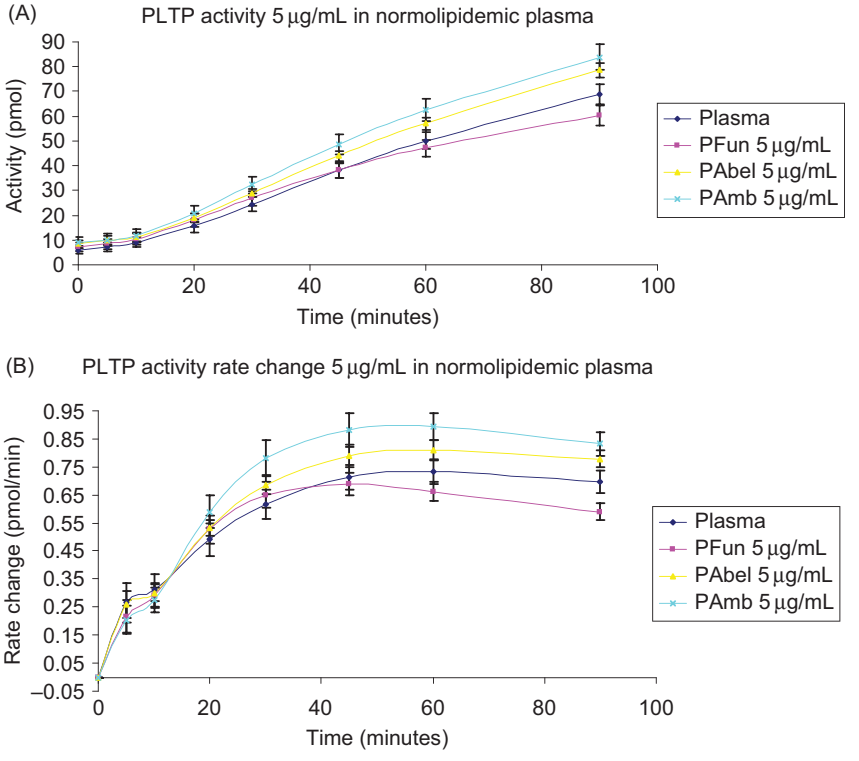

Figure 4. (A) Normolipidemic plasma that has been incubated at 37°C with 5 µg/mL Amp-B formulations. The graph illustrates the PLTP activity level at set time points of incubation (mean ± SEM, $n = 6$). (B) Normolipidemic plasma that has been incubated at 37°C with 1 µg/mL Amp-B formulations. The graph illustrates the PLTP activity rate of change at set time points of incubation from starting activity level (mean ± SEM, $n = 6$).

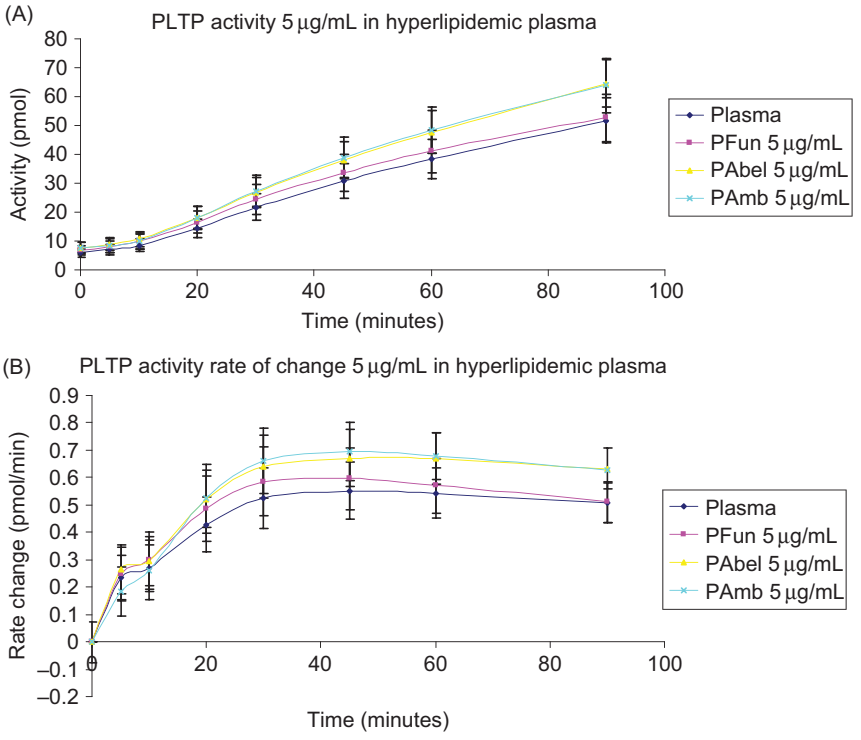

Figure 5. (A) Hyperlipidemic plasma that has been incubated at 37°C with 5 µg/mL Amp-B formulations. The graph illustrates the PLTP activity level at set time points of incubation (mean ± SEM, n=6). (B) Hyperlipidemic plasma that has been incubated at 37°C with 5 µg/mL Amp-B formulations. The graph illustrates the PLTP activity rate of change at set time points of incubation from starting activity level (mean ± SEM, n = 6).

The main differences found in this study come from data with the longest incubation time and highest drug addition to plasma.

At 90 minutes with 5 µg/mL of Amp-B addition to normolipidemic plasma, an increase in PLTP activity occurred from plasma baseline in Abelcet® (P < 0.01, +14.1%, +9.7 pmol; n = 6) and AmBisome® (P < 0.001, +21.8%, +15.03 pmol; n = 6), and a decrease occurred in Fungizone® (P < 0.01, –12.2%, –8.40 pmol; n = 6). The same pattern was also present in the PLTP activity rate of change over the 90 minutes of incubation (Abelcet®; P < 0.01, +11.6%; n = 6, AmBisome®; P < 0.001, +19.3%; n = 6, and Fungizone®; P < 0.001, –15.5%; n = 6, respectively).

At 90 minutes with 5 µg/mL of Amp-B addition to hyperlipidemic plasma, an increase in PLTP activity occurred from plasma baseline in Abelcet® (P < 0.001, +25.0%, +12.90 pmol; n = 6) and AmBisome® (P < 0.001, +23.7%, +12.23 pmol; n = 6), and a decrease occurred in Fungizone® (P > 0.05, +2.1%, +1.10 pmol; n = 6). The same pattern was also present in the PLTP activity rate of change over the 90 minutes of incubation (Abelcet®; P < 0.001, +24.3%; n = 6, AmBisome®; P < 0.001, +23.1%; n = 6, and Fungizone®; P > 0.05, +0.3%; n = 6, respectively).

Kinetics

The results for PLTP activity rate of change also allowed kinetic data to be analyzed on the effect of the Amp-B formulations on PLTP at a dose-dependent level.

Table 4 summarizes the pharmacokinetic data for Amp-B binding to PLTP at the 60-minute time point of incubation of which was chosen, as at this time point maximal effect was observed. Abelcet® and AmBisome® had an increased Vm over Fungizone® in both normolipidemic plasma and hyperlipidemic plasma but to a greater degree in normolipidemic plasma. In normolipidemic plasma, Abelcet® had a 30.9% (P < 0.01) increase over Fungizone® and AmBisome® had a 38.2% (P < 0.001) increase over Fungizone®. In hyperlipidemic plasma, Abelcet® had a 15.7% (P < 0.05) increase over Fungizone® and AmBisome® had a 16.9% (P < 0.01) increase over Fungizone®.

Discussion

Addition of Amp-B formulations was carried out with the belief that the lipid-complex formulation would show inhibition of PLTP as was seen in CETP. However,

Table 4. Comparison of Vm and Km values at each time point for Fungizone®, Abelcet®, and AmBisome® (at concentration levels of 1,2, and 5 μg/mL) in normolipidemic and hyperlipidemic plasma, $n=6$.

		60-minute time point			
		Normolipidemic plasma		Hyperlipidemic Plasma	
Fungizone®	Vm	0.66	(SD=0.089, SEM=0.036)	0.591	(SD=0.256, SEM=0.104)
Abelcet®	Vm	0.864	(SD=0.120, SEM=0.049)	0.684	(SD=0.220, SEM=0.090)
AmBisome®	Vm	0.912	(SD=0.912, SEM=0.048)	0.691	(SD=0.267, SEM=0.109)
Fungizone®	Km	−0.093	(SD=0.153, SEM=0.063)	0.085	(SD=0.100, SEM=0.041)
Abelcet®	Km	0.13	(SD=0.104, SEM=0.043)	0.186	(SD=0.129, SEM=0.053)
AmBisome®	Km	0.176	(SD=0.098, SEM=0.040)	0.149	(SD=0.089, SEM=0.036)

the results of these Amp-B additions showed that in normolipidemic plasma Fungizone® inhibited PLTP activity whereas Abelcet® and AmBisome® both increased PLTP activity. Additionally, in hyperlipidemic plasma Abelcet® and AmBisome® increased PLTP activity but Fungizone® showed no change to PLTP activity over that seen in plasma.

The results of this experiment show that PLTP activity is increased when lipid formulations of Amp-B (Abelcet® and AmBisome®) are added to human plasma. This result could be explained by the fact that one of PLTP's main functions is the transfer of phospholipids[22–24]. Thus, this observation would explain our previous finding by Patankar et al. where Abelcet® caused the preferential distribution on Amp-B into HDL fractions of plasma of which is seen to reduce nephrotoxicity. The slight differences in the effects of lipid type on PLTP also suggest that the lipid formulation itself has modifiable properties that could change its impact on PLTP activity. Furthermore, evidence of this was seen from the kinetic data which showed an increase in rate (Vm) for lipid Amp-B formulations over nonlipid Amp-B formulations but without change in affinity (Km). Thus, it suggests that the total lipids in the system are important for determining increased effects on PLTP activity as was also seen by reduced increases in PLTP activity and Vm as plasma changed from normolipidemic to hyperlipidemic states.

The changes found in this study represent importance in preventing nephrotoxicity in Amp-B patients as lipid formulations could be developed that further increase PLTP activity and thus increase Amp-B distribution into HDL. However, with PLTP in plasma being proatherogenic an increase in PLTP may have some harmful results, but this should be mitigated by the fact that Amp-B therapy itself is only usually given over a couple of weeks. Although the fact that Fungizione® decreased PLTP activity could lead to future studies as the molecular shape may have inhibitory properties on PLTP of which results over even less than a 10% change have been shown to result in significant benefits against atherosclerosis[25]. It may therefore be possible to formulate a drug that is inhibitory to PLTP activity and site direct it into HA-PLTP by coating the drug into a liposomal formulation.

In conclusion, this study has found that lipid-based Amp-B formulations work to increase PLTP activity and as such allow for the distribution of Amp-B on HDL fractions in plasma of which reduces the level of nephrotoxicity.

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Declaration of interest: The authors report no conflicts of interest.

References

1. Siggins S. (2005). Plasma phospholipid transfer protein (PLTP): quantitation, biosynthesis, and involvement in hepatic lipid homeostasis. National Public Health Institute - Kanasantervey-slaitos (KTL), September 16.
2. Tan KC, Shiu SW, Wong Y, Tam S. (2005). Plasma phospholipid transfer protein activity and subclinical inflammation in type 2 diabetes mellitus. Atherosclerosis, 178:365–70.
3. Lee-Rueckert M, Vikstedt R, Metso J, Ehnholm C, Kovanen PT, Jauhiainen M. (2006). Absence of endogenous phospholipid transfer protein impairs ABCA1-dependent efflux of cholesterol from macrophage foam cells. J Lipid Res, 47:1725–32.
4. Huuskonen J, Olkkonen VM, Jauhiainen M, Ehnholm C. (2001). The impact of phospholipid transfer protein (PLTP) on HDL metabolism. Atherosclerosis, 155:269–81.
5. Huuskonen J, Wohlfahrt G, Jauhiainen M, Ehnholm C, Teleman O, Olkkonen VM. (1999). Structure and phospholipid transfer activity of human PLTP: Analysis by molecular modeling and site-directed mutagenesis. J Lipid Res, 40:1123–30.
6. Cheung MC, Albers JJ. (2006). Active plasma phospholipid transfer protein is associated with apoA-I- but not apoE-containing lipoproteins. J Lipid Res, 47:1315–21.
7. Janis MT, Metso J, Lankinen H, Strandin T, Olkkonen VM, Rye KA, et al. (2005). Apolipoprotein E activates the low-activity form of human phospholipid transfer protein. Biochem Biophys Res Commun, 331:333–40.
8. van TA. (2002). Phospholipid transfer protein. Curr Opin Lipidol, 13:135–9.

9. Vikstedt R, Metso J, Hakala J, Olkkonen VM, Ehnholm C, Jauhiainen M. (2007). Cholesterol efflux from macrophage foam cells is enhanced by active phospholipid transfer protein through generation of two types of acceptor particles. Biochemistry, 46:11979–86.
10. Cheung MC, Brown BG, Marino Larsen EK, Frutkin AD, O'Brien KD, Albers JJ. (2006). Phospholipid transfer protein activity is associated with inflammatory markers in patients with cardiovascular disease. Biochim Biophys Acta, 1762:131–7.
11. Tall AR, Lalanne F. (2003). Phospholipid transfer protein and atherosclerosis. Arterioscler Thromb Vasc Biol, 23:1484–5.
12. Sakai N, Uchida Y, Ohashi K, Hibuse T, Saika Y, Tomari Y, et al. (2003). Measurement of fasting serum apoB-48 levels in normolipidemic and hyperlipidemic subjects by ELISA. J Lipid Res, 44:1256–62.
13. Jiang XC, Qin S, Qiao C, Kawano K, Lin M, Skold A, et al. (2001). Apolipoprotein B secretion and atherosclerosis are decreased in mice with phospholipid-transfer protein deficiency. Nat Med, 7:847–52.
14. Liu R, Hojjati MR, Devlin CM, Hansen IH, Jiang XC. (2007). Macrophage phospholipid transfer protein deficiency and ApoE secretion: Impact on mouse plasma cholesterol levels and atherosclerosis. Arterioscler Thromb Vasc Biol, 27:190–6.
15. Paszty C, Maeda N, Verstuyft J, Rubin EM. (1994). Apolipoprotein AI transgene corrects apolipoprotein E deficiency-induced atherosclerosis in mice. J Clin Invest, 94:899–903.
16. Schgoer W, Mueller T, Jauhiainen M, Wehinger A, Gander R, Tancevski I, et al. (2008). Low phospholipid transfer protein (PLTP) is a risk factor for peripheral atherosclerosis. Atherosclerosis, 196:219–26.
17. Sivak O, Lau B, Patankar N, Wasan KM. (2004). Unidirectional inhibition of lipid transfer protein I-mediated transfer of cholesteryl esters between high-density and low-density lipoproteins by amphotericin B lipid complex. Pharm Res, 21:2336–9.
18. Patankar N, Wasan KM. (2006). Role of phospholipid transfer protein on the plasma distribution of amphotericin B following the incubation of different amphotericin B formulations. Pharm Res, 23(5):1020–4.
19. Oka T, Kujiraoka T, Ito M, Nagano M, Ishihara M, Iwasaki T, et al. (2000). Measurement of human plasma phospholipid transfer protein by sandwich ELISA. Clin Chem, 46:1357–64.
20. Bekersky II, Fielding RM, Buell D, Lawrence II. (1999). Lipid-based amphotericin B formulations: From animals to man. Pharm Sci Technol Today, 2:230–6.
21. Bekersky I, Fielding RM, Dressler DE, Lee JW, Buell DN, Walsh TJ. (2002). Pharmacokinetics, excretion, and mass balance of liposomal amphotericin B (AmBisome) and amphotericin B deoxycholate in humans. Antimicrob Agents Chemother, 46:828–33.
22. Dullaart RP, De VR, Scheek L, Borggreve SE, Van GT, linga-Thie GM, et al. (2004). Type 2 diabetes mellitus is associated with differential effects on plasma cholesteryl ester transfer protein and phospholipid transfer protein activities and concentrations. Scand J Clin Lab Invest, 64:205–15.
23. linga-Thie GM, van TA, Hattori H, Rensen PC, Sijbrands EJ. (2006). Plasma phospholipid transfer protein activity is decreased in type 2 diabetes during treatment with atorvastatin: a role for apolipoprotein E? Diabetes, 55:1491–6.
24. O'Keefe Jr JH, Captain BK, Jones PG, Harris WS. (2004). Atorvastatin reduces remnant lipoproteins and small, dense low-density lipoproteins regardless of the baseline lipid pattern. Prev Cardiol, 7:154–60.
25. Murdoch SJ, Kahn SE, Albers JJ, Brunzell JD, Purnell JQ. (2003). PLTP activity decreases with weight loss: Changes in PLTP are associated with changes in subcutaneous fat and FFA but not IAF or insulin sensitivity. J Lipid Res, 44:1705–12.