Unidirectional Inhibition of Lipid Transfer Protein I–Mediated Transfer of Cholesteryl Esters Between High-Density and Low-Density Lipoproteins by Amphotericin B Lipid Complex

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Purpose. The purpose of this study was to determine whether Fungizone or amphotericin B lipid complex (ABLC; ABELCET®) affects the transfer of cholesteryl ester (CE) by lipid transfer protein I (LTP I; also known as cholesteryl ester transfer protein) between HDL and LDL (bidirectional transfer HDL to LDL and LDL to HDL).
Methods. Increasing concentrations of either Fungizone or ABELCET® (1.25–12.5 μg AmpB/ml) were incubated with HDL and [3H]CE-LDL or [3H]CE-HDL and LDL (the amount of each fraction added was equivalent to 10 μg of cholesterol) and LTP I in delipidated human plasma at 37°C for 90 min. As a positive control, TP2, a monoclonal antibody directed against LTP-1, was added instead of drug. After incubation, manganese and phosphate reagents were then added to precipitate out all of the LDL. The supernatant, consisted of only HDL, was counted for radioactivity to determine the amount of CE transferred from LDL. Similarly, the precipitate consisted of only LDL, was counted for radioactivity to determine the amount of CE transferred from HDL.
Results. For Fungizone, the transfer of cholesteryl ester (CE) between HDL and LDL were not significantly different compared to nontreated controls. For ABELCET®, CE transfer from HDL to LDL was significantly decreased at 12.5 μg AmpB/ml compared to control. However, transfer from LDL to HDL was not significantly different compared to non-treated controls. Similar results were observed with the major lipid component of ABELCET®, dimyristoylphosphatidylcholine. CE transfer from HDL to LDL and LDL to HDL was significantly decreased when using the positive control (TP2).
Conclusions. Fungizone does not affect LTP I–mediated transfer of CE between HDL and LDL. ABELCET® inhibits transfer from HDL to LDL, but has no effect on CE transfer from LDL to HDL. This uni-directional inhibition may contribute to the high recovery of AmpB in HDL but the very low presence of drug in the LDL fraction following ABELCET® incubation.

Key Words: Amphotericin B; amphotericin B lipid complex; lipid transfer protein I; lipoproteins.

INTRODUCTION

Lipid transfer protein I (LTP I) (1), also known as cholesteryl ester transfer protein, is a 476 amino acid glycoprotein with a molecular weight of 74,000. LTP I has been shown to be responsible for the facilitated transfer of core lipoprotein lipids cholesteryl esters (CE) and triglyceride (TG), coat lipoprotein lipid phosphatidylcholine (PC) transfer (2–4) and a portion of transfer of several water-insoluble drugs (5–7) between different plasma lipoprotein particles. Our laboratory has previously reported that LTP I facilitated transfer of one of these water-insoluble compounds, Amphotericin B (AmpB), from high-density lipoproteins (HDL) to low-density lipoproteins (LDL) (8). However, preliminary findings suggest that an increase in LTP I concentration increased the association of AmpB with serum LDL and decreased its association with the HDL/lipoprotein deficient serum fraction. This observation suggests that changes in LTP I concentration may regulate the distribution of AmpB among the HDL and LDL fractions of human serum. This notion is supported by work of Hughes *et al.* who hypothesized that the serum distribution of another water-insoluble compound which associates with serum lipoproteins, cyclosporine (CSA), was determined by factors other than simple diffusion between the lipoprotein particles (9). However, the increase in the concentration of LTP I did not increase the association of AmpB with serum LDL when ABELCET® [also known as amphotericin B lipid complex (ABLC); Enzon Pharmaceuticals Inc., Piscataway, NJ, USA] was incubated in human serum (8). Furthermore, the presence of empty or AmpB-containing lipid complexes decreased the ability of LTP I to transfer CE from HDL to LDL (8). These observations suggest that the presence of these lipid complexes in serum result in the reduction of LTP I-mediated transfer of CE from HDL to LDL. Because AmpB associates to unesterified and esterified cholesterol in serum (10), this finding may explain in part the decreased association of AmpB with serum LDL when formulated into these lipid complexes.

However, to date no studies have been done to determine if the presence of empty or AmpB-containing lipid complexes would modify the ability of LTP I to transfer CE from LDL to HDL. Because LTP I has the ability to transfer CE, TG, and phospholipids from both HDL to LDL and LDL to HDL but only has the ability to transfer AmpB from HDL to LDL, determining if AmpB-containing lipid complexes would decrease LTP I–mediated transfer of CE from LDL to HDL would complete the story. Because the majority of AmpB was recovered in the HDL fraction following the incubation of ABELCET® in plasma, we hypothesized that the presence of ABLC would decrease LTP I–mediated transfer of CE from HDL to LDL, but not from LDL to HDL. Information from this study may provide additional evidence to explain why the majority of AmpB remains with the HDL fraction following the incubation of ABELCET® in plasma.

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ABBREVIATIONS: ABLC, amphotericin B lipid complex; LTP I, lipid transfer protein I; CE, cholesteryl ester; TG, triglyceride; AmpB, amphotericin B; HDL, high-density lipoproteins; LDL, low-density lipoproteins; TRL, triglyceride-rich lipoproteins; TP2, monoclonal antibody directed against lipid transfer protein I; LPDP, lipoprotein deficient plasma; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate buffered saline; CMC, carboxy-methylcellulose; PC, egg phosphatidylcholine; k, constant, fraction of label transferred; t, time; DMPC, dimyristoylphosphatidylcholine; DMPG, dimyristoylphosphatidylglycerol.

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MATERIALS AND METHODS

Chemicals and Plasma

Radiolabeled CE ([1α,2α (n)-^{3}H] Cholesteryl oleate; Specific Activity, 71.9 mCi/mg) was purchased from Amersham Life Science (Buckinghamshire England). Sodium bromide was purchased from Sigma Chemical Company (St. Louis, MO, USA). Normolipidemic fasted human plasma was obtained from the Bioreclamation (East Meadow, NY, USA). Ten μl of 0.4 M ethylenediaminetetraacetic acid pH 7.1 (EDTA, Sigma Chemical Company, St. Louis, MO) was added to 1.0 ml of whole blood. Fungizone (Bristol Myers Squibb, Nutley, NJ, USA) and ABELCET® (ABLC; Enzon Pharmaceuticals Inc., Piscataway, NJ, USA) was purchased from the Vancouver General Hospital Department of Pharmacy Services (Vancouver, BC, Canada). ABELCET® consists of AmpB combined with two phospholipids in a roughly a 1:1 drug-to-lipid weight ratio. Each ml of ABELCET® suspension contains 5 mg of AmpB USP, 3.4 mg of DMPC, and 1.5 mg of DMPG.

Lipoprotein Separation

The plasma was separated into its HDL, LDL, VLDL, and lipoprotein deficient plasma (LPDP) fractions by ultracentrifugation (11,12).

Radiolabeling of Plasma Lipoproteins

Human HDL and LDL were labeled by the lipid dispersion technique as previously described (2,3). High-density lipoproteins (HDL) labeled with ^{3}H-CE had a specific activity of 1.9×10^{-3} μCi /10 μg HDL cholesterol while low-density lipoproteins labeled with ^{3}H-CE had a specific activity of 1.6×10^{-3} μCi /μg LDL cholesterol.

Lipid Transfer Assays

Lipid (CE) transfers were performed within lipoprotein-deficient plasma as has been previously described (2,8,13). Typically, 10 μg (total cholesterol) of radiolabeled donor and unlabeled acceptor are incubated ± LTP I (1.0 μg protein/ml; concentration was determined from a dose response curve; data not shown) in delipidated human plasma (delipidated human plasma was used as a LTP I source with a concentration of 1.0 μg protein/ml as determined by ELISA), pH 7.4 for 90 min (time was determined from a time response curve; data not shown) at 37°C . Lipid transfer between donor and acceptor lipoprotein is then quantitated by scintillation counting. The fraction of lipid transferred (kt) is calculated as described by Pattnaik and Zilversmit (13):

$$kt = -\ln (1 - A_t/D_0)$$

where D_o and A_t are the radioactivities in the donor at time 0 and in the acceptor at time t, respectively. The constant k is the fraction of label transferred per unit time (t). Acceptor radioactivity in the absence of LTP I (usually <2–3%) is subtracted before calculating kt values. Calculations assume steady-state conditions where all lipid transfer is an exchange process.

Quantification of Plasma Lipids and Amphotericin B

Enzymatic assay kits from Sigma Diagnostics (St. Louis MO, USA) determined total and lipoprotein triglyceride and cholesterol concentrations. AmpB levels in lipoprotein and lipoprotein-deficient fractions were analyzed by high-pressure liquid chromatography (HPLC) as previously described (8).

Experimental Design

To determine whether Fungizone or ABELCET® affects the transfer of CE by LTP I between HDL and LDL (bidirectional transfer HDL to LDL and LDL to HDL), increasing concentrations of either Fungizone or ABELCET® (1.25–12.5 μg AmpB/ml) or DMPC (12.5 μg DMPC/ml) were incubated with HDL and [3H]CE-LDL or [3H]CE-HDL and LDL (the amount of each fraction added was equivalent to 10 μg of cholesterol) and the LTP I source (delipidated plasma) at 37°C for 90 min. After incubation manganese and phosphate reagents were then added to precipitate out all of the LDL. The supernatant, consisted of only HDL, was counted for radioactivity to determine the amount of [3H]CE transferred from LDL. Similarly, the precipitate, consisted of only LDL, was counted for radioactivity to determine the amount of [3H]CE transferred from HDL. To confirm that the transfer of CE is due to LTP I and not other endogenous plasma factors, TP2 (8-μg protein/ml; as determined by ELISA) (data not shown) was co-incubated with CE-enriched and -free lipoprotein particles in plasma (Table I).

To determine the AmpB plasma distribution following incubation of either Fungizone or ABELCET® in human plasma, 20 μg AmpB/ml of either formulation was incubated in human plasma for 90 min at 37°C. Following incubation, the plasma was separated into its different lipoprotein and lipoprotein deficient fractions by density gradient ultracentrifugation and analyzed for AmpB against an external calibration curve by HPLC as previously described (8).

Table I. Percent Transfer of Cholesteryl Ester (CE) Between Lipoproteins, in the Presence or Absence of Fungizone, Amphotericin B Lipid Complex (ABLC), Blank Liposomes (DMPC), and a Monoclonal Antibody (TP2) Directed Against LTP I in Human Plasma [LTP I Source is Human Plasma (1 μg Protein/ml)]

Treatment	Concentration (μg/ml)	HDL to LDL (%kt)[a]	LDL to HDL (%kt)[a]
Control	—	25.5 ± 1.7	22.1 ± 4.3
TP2 (protein)	8 μg protein/ml	9.6 ± 3.1*	8.8 ± 3.7*
DMPC liposomes	12.5 μg DMPC/ml	18.1 ± 1.1*	30.1 ± 1.8
Fungizone (AmpB)	1.25 μg AmpB/ml	25.1 ± 1.4	23.0 ± 2.1
	6.25 μg AmpB/ml	21.5 ± 1.3	18.4 ± 5.1
	12.5 μg AmpB/ml	22.1 ± 1.0	26.0 ± 3.8
Abelcet (ABLC)	1.25 μg AmpB/ml	23.0 ± 0.5	28.6 ± 2.9
	6.25 μg AmpB/ml	22.1 ± 1.1	25.7 ± 1.9
	12.5 μg AmpB/ml	17.5 ± 1.0*	30.5 ± 5.6

Data presented as mean ± standard deviation; n = 8 for control and TP2 and n = 6 for DMPC liposomes, fungizone, and amphotericin B lipid complex (ABLC).
* $p < 0.05$ vs. control using PCANOVA.

Statistical Analysis

Differences in LTP I mediated [3H]CE transfer activity and AmpB plasma distribution in the of different formulations were determined by a two-way analysis of variance (PCANOVA; Human Systems Dynamics). Critical differences were assessed by Neuman-Keuls *post hoc* tests. Differences were considered significant if p was <0.05. All data are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The objective of this study was to determine whether Fungizone or ABELCET® affects the transfer of cholesteryl ester (CE) by lipid transfer protein I (LTP I; also known as cholesteryl ester transfer protein) between HDL and LDL (bidirectional transfer HDL to LDL and LDL to HDL). Our data suggests that Fungizone does not affect LTP I–mediated transfer of CE between HDL and LDL (Table I). However, ABELCET® at only a concentration of 12 μg AmpB/ml significantly decreases CE transfer from HDL to LDL. ABELCET® does not affect LTP I–mediated transfer of CE from LDL to HDL compared to controls (Table I).

We have previously demonstrated that the distribution of AmpB between HDL and LDL following incubation in human plasma is facilitated by LTP I. However, once AmpB was incorporated into lipid complexes and liposomes composed of negatively charged and neutral phospholipids, the ability of LTP I to transfer AmpB and ^{3}H-CE from HDL to LDL diminished (8,14). We concluded from these studies that because AmpB interacts with free cholesterol and CE upon incubation in plasma (10,14), LTP I's ability to transfer AmpB between HDL and LDL was due to its ability to transfer CE between HDL and LDL and not due to the direct transfer of AmpB between lipoprotein fractions.

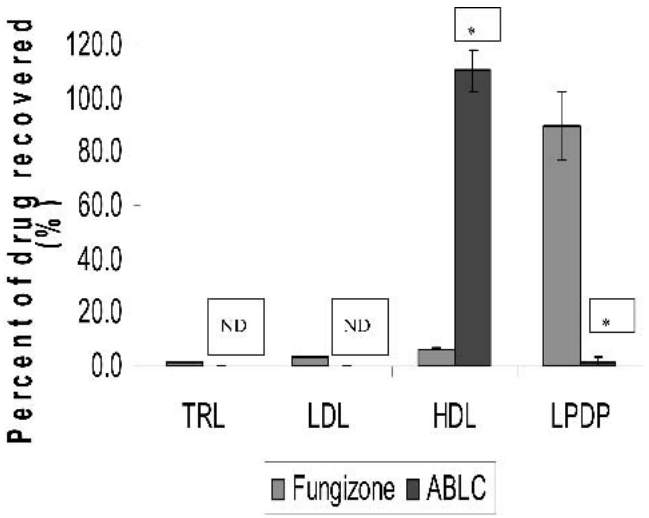

Fig. 1. Plasma distribution of AmpB following incubation of Fungizone or ABELCET® (ABLC) at 20 μg AmpB/ml for 90 min at 37 C. *p < 0.05 vs. Fungizone. Data presented as mean ± standard deviation (n = 6). Abbreviations: AmpB, amphotericin B, TRL, triglyceride-rich lipoproteins (which consists of very-low density lipoproteins and chylomicrons); HDL, high-density lipoproteins; LDL, low-density lipoproteins; LPDP, lipoprotein-deficient plasma (which consists of albumin and alpha-1-glycoprotein); ABLC, amphotericin B lipid complex; ND, non-detectable, below the limit of the HPLC assay.

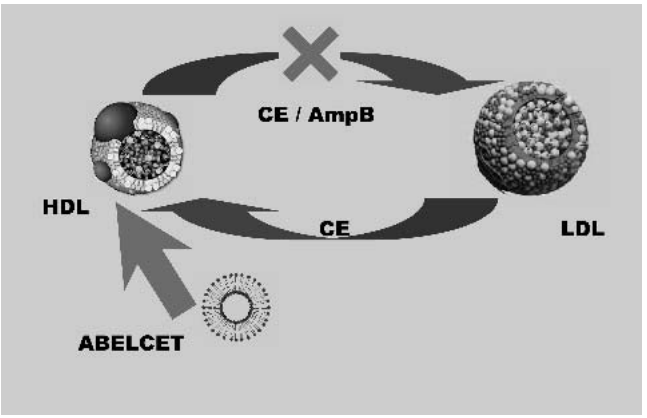

Fig. 2. ABELCET® exhibits a unidirectional inhibition of LTP I–mediated CE and AmpB transfer by decreasing transfer from HDL to LDL but not from LDL to HDL. Abbreviations: LTP I, lipid transfer protein I; CE, cholesteryl esters; AmpB, amphotericin B; HDL, high-density lipoproteins; LDL, low-density lipoproteins.

Because LTP I can transfer CE from LDL to HDL as well as from HDL to LDL investigating only the effects of LTP I–mediated transfer of CE from HDL to LDL in our previous study was incomplete (8). Findings from this study suggest that ABELCET® exhibits a unidirectional inhibition of LTP I–mediated CE transfer by decreasing transfer from HDL to LDL but not from LDL to HDL. Because AmpB, when formulated into ABELCET® is mainly distributed into HDL fraction (Fig. 1), we hypothesize that the phospholipids from the formulation (Table I) (8) interferes with LTP I's ability to access CE and AmpB within HDL. This results in a decreased LTP I–mediated transfer of CE and AmpB from HDL to LDL (Fig. 2). However, AmpB, when formulated into ABELCET®, does not significantly distribute into the LDL fraction (Fig. 1), therefore, interference of LTP I by phospholipids is not seen, and CE present in LDL is available for transport by LTP I to HDL (Fig. 2).

In conclusion, we have found that Fungizone does not affect LTP I–mediated transfer of CE between HDL and LDL. In contrast, ABELCET® significantly decreases CE transfer from HDL to LDL, but has no effect on transfer from LDL to HDL. This unidirectional inhibition may be a partial explanation to why a high concentration of AmpB is recovered in HDL and very low presence of AmpB is recovered in the LDL fraction following the administration of ABELCET®.

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