Differences in the Lipoprotein Distribution of Halofantrine Are Regulated by Lipoprotein Apolar Lipid and Protein Concentration and Lipid Transfer Protein I Activity: In Vitro Studies in Normolipidemic and Dyslipidemic Human Plasmas

KISHOR M. WASAN,*,† MANISHA RAMASWAMY,† MICHELLE P. MCINTOSH,‡ CHRISTOPHER J. H. PORTER,‡ AND WILLIAM N. CHARMAN‡

Contribution from *Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences, The University of British Columbia, 2146 East Mall, Vancouver, British Columbia, Canada V6T 1Z3, and Victorian College of Pharmacy, Monash University, Parkville, Victoria, Australia, 3052.*

Received August 27, 1998. Final revised manuscript received November 4, 1998.
Accepted for publication November 9, 1998.

Abstract □ The purpose of these studies was to determine the distribution of a lipophilic antimalarial agent, halofantrine hydrochloride (Hf), in fasted plasma from hypo-, normo-, and hyperlipidemic patients that displayed differences in lipoprotein concentration and lipid transfer protein I (LTP I) activity. To assess the influence of modified lipoprotein concentrations and LTP I activity on the plasma distribution of Hf, Hf at a concentration of 1000 ng/mL was incubated in either hypo-, normo-, or hyperlipidemic human plasma for 1 h at 37 °C. Following incubation, the plasma samples were separated into their lipoprotein and lipoprotein-deficient plasma (LPDP) fractions by density gradient ultracentrifugation and assayed for Hf by high-pressure liquid chromatography. The activity of LTP I in the dyslipidemic plasma samples was determined in terms of its ability to transfer cholesteryl ester from low-density lipoproteins (LDL) to high-density lipoproteins (HDL). Total plasma and lipoprotein cholesterol (esterified and unesterified), triglyceride, and protein levels in the dyslipidemic plasma samples were determined by enzymatic assays. When Hf was incubated in normolipidemic plasma for 1 h at 37 °C, the majority of drug was found in the LPDP fraction. When Hf was incubated in human plasma of varying total lipid, lipoprotein lipid, and protein concentrations and LTP I activity, the following relationships were observed. As the triglyceride-rich lipoprotein (TRL) lipid and protein concentration increased from hypolipidemia through to hyperlipidemia, the proportion of Hf associated with TRL increased ($r > 0.90$). As the HDL lipid and protein concentration increased, the proportion of Hf associated with HDL decreased ($r > 0.70$). As the total and lipoprotein lipid levels increased, the LTP I activity of the plasma also proportionally increased ($r > 0.85$). Furthermore, with the increase in LTP I activity, the proportion of Hf associated with the TRL fraction increased ($r > 0.70$) and the proportion of Hf associated with the HDL fraction decreased ($r > 0.80$). In addition, a positive correlation between the proportion of apolar lipid and Hf recovered within each lipoprotein fraction was observed within hypo- ($r > 0.80$), normo- ($r = 0.70$), and hyperlipidemic ($r > 0.90$) plasmas. These findings suggest that changes in the HDL and TRL lipid and protein concentrations, LTP I activity, and the proportion of apolar lipid within each lipoprotein fraction may influence the plasma lipoprotein distribution of Hf in dyslipidemia.

Introduction

Lipoproteins are macromolecules of lipid and protein that transport lipids through the vascular and extravascular bodily fluids.[1] Great diversity in the composition and physical properties of lipoproteins are possible, particularly in disease states. However, it is becoming apparent that lipoproteins have a wider biological significance than simply in lipid transport. It has been demonstrated that the interaction of several compounds, including amphotericin B (AmpB)[2,3] and cyclosporine (CSA),[4] with plasma lipoproteins modifies the pharmacokinetics, tissue distribution, and pharmacologic activity of these compounds. In addition, recent studies have suggested that not only the relative levels of individual lipoproteins but also their lipid composition define the distribution of a number of hydrophobic compounds among plasma lipoproteins.[5−7]

Halofantrine (Hf), an effective agent in the treatment of malaria, has been shown to bind to lipoproteins upon incubation in human blood[8] and plasma.[9] Cenni et al.[8] have suggested that Hf interacts and binds mostly to low-density lipoproteins (LDL) and high-density lipoproteins (HDL) upon incubation in human blood from noninfected and malaria subjects. However two drawbacks of this work were that the Hf distribution experiments were not done at a physiologic temperature, but rather at 20 °C, and that the total Hf recovery from plasma was as low as 40%. Humberstone et al.[10] have demonstrated that the lipoprotein distribution of Hf within fasted and fed beagle plasma samples may be influenced by the relative pre- and post-prandial lipoprotein profiles. Furthermore, McIntosh et al.[9] have recently concluded that these differences are regulated by the respective masses of core apolar lipoprotein lipid.

Recent studies have suggested that the binding of Hf to lipoproteins may also alter its pharmacokinetics and pharmacological effect. Humberstone et al.[10] have observed, following intravenous Hf administration to fasted and fed beagles, that a decrease in Hf clearance and a decrease in Hf volume of distribution was correlated with an increased binding of Hf to plasma lipoproteins in the post-prandial state compared with the fasted state. In addition, they demonstrated that the concentration that is inhibitory in 50% of test subjects (IC_{50}) of Hf within an in vitro culture of *Plasmodium falciparum* was significantly decreased when incubated in the presence of 10% post-prandial serum.[11] Taken together, these studies suggest that the effect of human dylipidemias (aberrant plasma lipoprotein and lipid concentrations) on the plasma distribution of Hf merit further investigation.

Most patients infected with malaria may exhibit disturbances in their lipid metabolism resulting in modified lipoprotein−lipid composition,[8] so we conducted studies to

* Corresponding author. Telephone: 604-822-4889. Fax: 604-822-3035. E-mail: Kwasan@unixg.ubc.ca.
† Division of Pharmaceutics and Biopharmaceutics.
‡ Victorian College of Pharmacy.

10.1021/js980353k CCC: $18.00
Published on Web 01/05/1999

determine whether the human plasma distribution of Hf was influenced by changes in lipoprotein concentration and composition. Our working hypothesis was that the association of Hf with different lipoprotein classes would be influenced by the relative abundance of these lipid−protein complexes and apolar lipid content. In addition, because Hf is lipophilic, we believed that lipid transfer protein I (LTP I), an endogenous glycoprotein responsible for the transfer of lipids between lipoproteins,[12] may influence Hf lipoprotein distribution in a similar manner as it regulates the transfer of AmpB[13] and CSA[14] between HDL and LDL.

Materials and Methods

Chemicals and Plasma—Halofantrine was provided by Smith-Kline Beecham Pharmaceuticals (King of Prussia, PA). Sodium bromide was purchased from Sigma Chemical Company (St. Louis, MO). Liquid chromatography grade acetonitrile and *tert*-butyl methyl ether (TBME) were obtained from Fisher Canada (Montreal, Quebec). Electrophoresis grade sodium dodecyl sulfate (SDS) was obtained from Eastman Kodak (Rochester, NY). Fasted human plasma samples from hypolipidemic, normolipidemic, and a hyperlipidemic subjects were obtained from the Vancouver Red Cross (Vancouver, British Columbia). Ten microliters of 0.4 M ethylenediaminetetraacetic acid, pH 7.1 (EDTA; Sigma Chemical Company) was added to 1.0 mL of whole blood. In this study, a hypolipidemic plasma sample was defined as having a total plasma cholesterol of <130 mg/dL and total plasma triglyceride of <100 mg/dL, a normolipidemic plasma sample was defined as having a total plasma cholesterol of 130−200 mg/dL and total plasma triglyceride of 100−200 mg/dL, and a hyperlipidemic plasma sample was defined as having a total plasma cholesterol of >350 mg/dL and total plasma triglyceride of >280 mg/dL.[1] For all Hf plasma distribution studies, Hf was dissolved in 100% methanol. The addition of methanol alone did not modify lipoprotein−lipid composition or plasma LTP I activity (data not shown).

Lipoprotein Separation—The plasma was separated into its HDL, LDL, triglyceride-rich lipoproteins (TRL), which consists of VLDL and chylomicrons, and lipoprotein deficient plasma (LPDP) fractions by ultracentrifugation.[15,16] Briefly, human plasma (3.0 mL) samples were placed in centrifuge tubes and their solvent densities were adjusted to 1.006 g/mL by sodium bromide. Following centrifugation (L8−80M; Beckman Canada) at 50 000 rpm for 18 h at 4 °C, the TRL-containing and TRL-deficient plasma fractions were recovered. The TRL-deficient plasma fraction was readjusted to a density of 1.063 g/mL and respun at 50 000 rpm for 18 h at 4 °C to separate the LDL-rich and TRL/LDL-deficient plasma fractions. This fraction was readjusted to a density of 1.21 g/mL and respun at 50 000 rpm for 18 h at 4 °C to separate the HDL-rich and lipoprotein-deficient plasma (LPDP) fractions.

Isolation and Purification of Lipid Transfer Protein (LTP I)—LTP I was purified from human lipoprotein-deficient plasma as has been previously described.[12] Briefly, citrated human plasma was made lipoprotein deficient by the dextran−$MnCl_2$ procedure of Burstein and co-workers.[17] LTP I was then partially purified by sequential chromatography on phenyl-Sepharose and carboxy methyl cellulose gel (CM-52, Whatman Inc., Chifton, NJ). Partially purified LTP I (1.05 mg protein/mL), enriched 800-fold relative to lipoprotein-deficient plasma, was stored at 4 °C in 0.01% disodium EDTA, pH 7.4. The CM-cellulose fraction of LTP I was used in all experiments.

Radiolabeling of Plasma Lipoproteins—Human LDL was labeled by the lipid dispersion technique as previously described.[12] Briefly, human plasma was incubated with a lipid dispersion containing egg phosphatidylcholine (PC), triglyceride (5 mol %), and [^{3}H]cholesteryl ester (CE) (12 mol %) at 37 °C for 20−24 h in the presence of LTP I and diethyl (*p*-nitrophenyl) phosphate. Then the LDL fractions were isolated from the total lipoprotein precipitate by centrifugation as previously described. LDL had a specific activity of 3.423×10^6 cpm/mL (1352 cpm/μg of total cholesterol).

Lipid Transfer Assays—To assess the LTP I activity within the different human plasma samples used in this study, the following protocol was used. Lipid (CE) transfer was performed within the lipoprotein-deficient plasma samples as has been previously described.[14,18] Typically, 10 μg (total cholesterol) of radiolabeled donor (^{3}H−CE LDL) and unlabeled acceptor (HDL) are incubated ± LTP I source (which are the different delipidated human plasma samples used in this study), pH 7.4, for 90 min at 37 °C. Lipid transfer between donor and acceptor lipoprotein is then quantitated by scintillation counting. The fraction of lipid and drug transferred (kt) was calculated as described by Pattnaik and Zilversmit:[19]

$$k_t = -\ln(1 - A_t/D_0) \quad (1)$$

where D_0 and A_t are the amounts of radioactivity 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 (usually < 2−3%) is subtracted before calculating k_t values. Calculations assume steady-state conditions, where all lipid transfer is an exchange process. To minimize calculation errors due to mass transfer, all values were determined from assays in which the extent of radiolabel transfer was small (≤15%).

Quantification of Halofantrine—Plasma lipoprotein and lipoprotein-deficient samples containing Hf (1-mL aliquot) were mixed with 2 mL of acetonitrile and 8 mL of TBME; the mixture was vortexed for 30 s after the addition of acetonitrile and TBME and centrifuged (2000x *g* for 2 min). The supernatant was transferred to another tube, dried under nitrogen at 30 °C, and reconstituted with 0.5 mL of acetonitrile. The reconstituted extractant was analyzed against an external standard calibration curve for Hf by a high-pressure liquid chromatography (HPLC) procedure developed by Humberstone and co-workers.[20] The internal standard used to account for variation between samples is 2,4-dichloro-6-trifluoromethyl-9-{1-(2-(dibutylamino)ethyl)}-phenanthrenemethanol.[20] The HPLC system consisted of a Beckman model 110A pump, a Shimadzu SIL-9A autoinjector, and a Shimadzu SPD-6A variable wavelength detector. The detector was set at a wavelength of 257 nm, with absorbance sensitivity of 0.05 absorbency units, full scale. Results were recorded on a Shimadzu C−R3A chromatopac integrator. For chromatographic separation, a ultrasphere ODS column (4.6 mm × 25 cm) packed with trimethylsilyl particles (particles of 5 μm in diameter) was used at ambient temperatures. The mobile phase consisted of 75:25 (v/v) acetonitrile:water with 0.2% (w/v) SDS and 0.2% (V/v) glacial acetic acid at a flow rate of 1.5 mL min^{-1}. This assay had a limit of quantitation for Hf of 39 ng/mL and external calibration curves in total plasma, lipoprotein, and lipoprotein-deficient fractions that were linear in a concentration range of 39−5000 ng/mL of Hf (r > 0.90), with an intraday coefficient of variation of 5−8%.

Determination of Plasma Lipoprotein Triglyceride, Cholesterol, and Protein Concentrations—Total plasma triglycerides (TG), cholesterol, and protein concentrations were determined by enzymatic assays purchased from Sigma Diagnostics (St. Louis Mo.) as previously described.[17] The external calibration curve for plasma and lipoprotein triglyceride was linear in a concentration range 10−300 mg/dL (r > 0.95), for cholesterol was linear in a concentration range 10−450 mg/dL (r > 0.96), and for protein was linear in a concentration range of 5−2000 mg/dL (r > 0.90). Free cholesterol was determined using an enzymatic assay purchased from Boehringer Mannheim and was linear in a concentration range 1−100 mg/dL (r > 0.90). Cholesteryl ester concentration was determined by calculating the difference between total and free cholesterol.

Halofantrine (Hf) Distribution Studies in Specific Human Plasma Samples of Varying Lipoprotein Concentration and Composition and LTP I Activity—To gain an understanding on how lipoprotein lipid and protein concentration and LTP I influences the plasma distribution of Hf, Hf (1000 ng/mL) was incubated in 3 mL of plasma samples (total amount of Hf in plasma was 3000 ng) from three different patients (hypo-, normo-, and hyperlipidemic) of varying total and lipoprotein cholesterol, triglyceride, and protein concentrations (Table 1) and composition (Table 2) for 60 min at 37 °C. A Hf concentration of 1000 ng/mL was chosen as a value broadly representative of serum concentrations expected in a clinical enviroment.[21] Following incubation, the plasma was cooled to 4 °C to prevent any drug redistribution (data not shown) and separated into its lipoprotein and lipoprotein-deficient fractions by density gradient ultracentrifugation, and each fraction was quantified for Hf by HPLC.[20] To ensure that the distribution of Hf found in each of these fractions was a result of its association with each lipoprotein or lipoprotein-deficient

Table 1—Fasted Total and Lipoprotein Plasma Cholesterol (Esterified + Unesterified), Triglyceride, and Protein Concentrations in Plasma Samples from Three Individual Patients[a]

patient profile	TRL, mg/dL	LDL, mg/dL	HDL, mg/dL	total, mg/dL
		cholesterol (esterified + unesterified)		
hypolipidemic	20.6 ± 2.6	70.8 ± 3.1	32.3 ± 1.7	123.7 ± 5.6
normolipidemic	43.9 ± 1.8*	108.2 ± 9.9*	44.7± 6.1*	196.9 ± 4.0*
hyperlipidemic	112.6 ± 9.4*	180.7 ± 16.1*	105.2 ± 13.9*	398.5 ± 18.1*
		triglyceride		
hypolipidemic	31.1± 2.1	22.7 ± 1.1	21.4 ± 3.4	75.1 ± 2.1
normolipidemic	92.1 ± 8.5*	45.3 ± 7.8*	33.0 ± 3.6*	170.4 ± 4.8*
hyperlipidemic	153.5 ± 16.9*	80.3 ± 11.3*	61.4 ± 6.6*	295.1 ± 18.6*
		protein		
hypolipidemic	7.4 ± 1.2	25.0 ± 2.3	132.4 ± 11.8	164.7 ± 11.8
normolipidemic	34.8 ± 2.8*	94.4 ± 2.8*	217.6 ± 9.0*	346.8 ± 10.2*
hyperlipidemic	77.9 ± 6.8*	156.3 ± 11.1*	936.9 ± 111*	1171 ± 115*

[a] Data are expressed as mean ± standard deviation ($n = 4$ replicates for hypolipidemic; $n = 6$ replicates for normolipidemic and hyperlipidemic). Abbreviations: TRL, triglyceride rich lipoproteins, which includes very low-density lipoproteins and chylomicrons; LDL, low-density lipoproteins; HDL, high-density lipoproteins. (*) $p < 0.05$ versus hypolipidemic patient profile.

Table 2—Lipoprotein Composition of Plasma from Hypolipidemic, Normolipidemic, and Hyperlipidemic Patients[a]

lipoprotein fraction	hypolipidemic	normolipidemic	hyperlipidemic
	triglyceride rich lipoproteins		
TC/TP (wt/wt)	2.8 ± 0.3	1.2 ± 0.1*	1.5 ± 0.2*
TG/TP (wt/wt)	4.3 ± 1.0	2.5 ± 0.1*	2.0 ± 0.3* **
TG/TC (wt/wt)	2.2 ± 0.4	2.9 ± 0.1*	1.4 ± 0.1* **
	low-density lipoproteins		
TC/TP (wt/wt)	2.9 ± 0.3	1.2 ± 0.1*	1.2 ± 0.1*
TG/TP (wt/wt)	0.9 ± 0.1	0.5 ± 0.1*	0.5 ± 0.1*
TG/TC (wt/wt)	0.30 ± 0.03	0.4 ± 0.1	0.44 ± 0.05*
	high-density lipoproteins		
TC/TP (wt/wt)	0.25 ± 0.02	0.13 ± 0.03*	0.12 ± 0.03*
TG/TP (wt/wt)	0.16 ± 0.01	0.11 ± 0.02*	0.07 ± 0.01* **
TG/TC (wt/wt)	0.66 ± 0.06	0.87 ± 0.17	0.59 ± 0.07**

[a] Data are expressed as mean ± standard deviation ($n = 4$ replicates for hypolipidemic and $n = 6$ replicates for normolipidemic and hyperlipidemic); (*) $p < 0.05$ versus hypolipidemic; (**) $p < 0.05$ versus normolipidemic; TC, total cholesterol (esterified + unesterified); TG, total triglycerides; TP, total protein; wt/wt, weight/weight; plasma type, hypolipidemic (total cholesterol <130 mg/dL and triglyceride < 100 mg/dL); normolipidemic (total cholesterol 130–200 mg/dL and triglyceride 100–200 mg/dL); hyperlipidemic (total cholesterol > 350 mg/dL and triglyceride > 280 mg/dL).

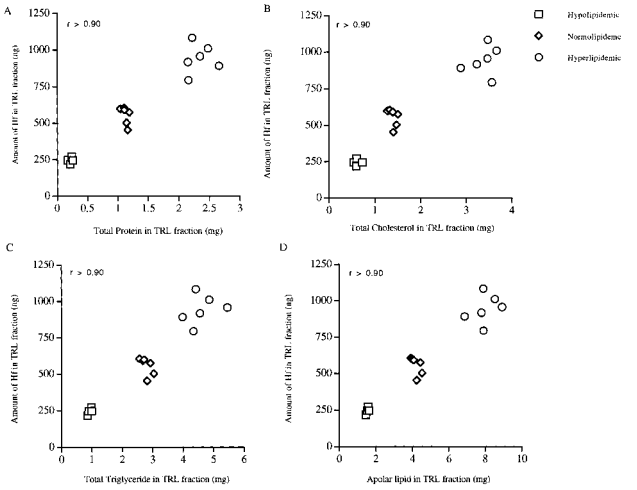

Figure 1—Distribution of Hf at 1000 ng/mL incubated for 60 min at 37 °C in hypolipidemic, normolipidemic, or hyperlipidemic plasma samples: (A) triglyceride-rich lipoprotein (TRL) protein; (B) TRL cholesterol, (C) TRL TG; and (D) TRL apolar lipid (cholesteryl ester and triglyceride) content versus the amount of Hf within the TRL fraction.

fraction and not a result of the density of the drug, the density profile of Hf reconstituted in 100% methanol and incubated in lipoprotein-deficient plasma was determined by ultracentrifugation. The majority of Hf (>85%) was found in the density range >1.21 g/mL (data not shown), suggesting that the Hf distribution within the ultracentrifuge tubes following incubation in human plasma is not a function of drug density.

In addition, to provide further evidence that LTP I may facilitate the movement of Hf between lipoprotein fractions, the lipoprotein distribution of Hf within normolipidemic human plasma (containing 1.0 μg protein/mL of LTP I), which has been supplemented with exogenous LTP I (0.67 μg protein/mL), was determined.

Data and Statistical Analysis—Correlation coefficients between the amount of Hf recovered within the TRL, HDL, and LDL plasma fractions and the amount of cholesterol, triglyceride, and protein within these fractions (Figures 1–3), proportion of apolar lipid within each lipoprotein fraction (Figure 4), and LTP I activity (Figure 5) were determined using Pearson's test. Differences in the human plasma distribution of Hf following incubation in human plasmas of varying lipid and protein concentrations were determined by a two-way analysis of variance (PCANOVA; Human Systems Dynamics). Critical differences were assessed by Neuman–Keuls posthoc tests. Differences were considered significant if p was <0.05. All data are expressed as mean ± standard deviation.

Results and Discussion

Total and Lipoprotein Lipid and Protein Levels in Plasma from Human Subjects—The differences in total and lipoprotein plasma cholesterol (esterified + unesterified), triglyceride, and protein concentrations and composition between hypolipidemic, normolipidemic, and hyperlipidemic human subjects chosen for this study are reported in Tables 1 and 2.

Distribution of Halofantrine (Hf) Following Incubation in Plasma from Human Subjects with Varying Lipid Concentrations—Table 3 reports the plasma distribution of Hf at 1000 ng/mL within plasma obtained from hypolipidemic, normolipidemic, and hyperlipidemic human subjects following incubation for 60 min at 37 °C. A significantly greater percentage of Hf was recovered within the TRL fraction following incubation in hyperlipidemic and normolipidemic patient plasma for 60 min at 37 °C than following incubation in hypolipidemic patient plasma. At the same time, a significantly lower percentage of Hf was recovered within the HDL fraction following incubation in hyperlipidemic and normolipidemic patient plasma samples than following the incubation in hypolipidemic patient plasma samples. In addition, a significantly higher percentage of Hf was recovered within the TRL fraction and significantly lower percentage of Hf was recovered in the HDL and LPDP fractions following incubation in hyperlipidemic compared with normolipidemic patient plasma samples.

Table 3—Distribution of Halofantrine Hydrochloride (Hf·HCl) at 1000 ng/mL within Fasted Plasma from Three Individual Patients Following Incubation for 60 min at 37 °C (following incubation plasma samples were assayed by high-pressure liquid chromatography for drug in each of the lipoprotein and lipoprotein-deficient plasma fractions)[a]

patient profile	TRL fraction, %[b]	LDL fraction, %	HDL fraction, %	LPDP fraction, %	percent recovery[c]	LTP I activity, %k_t
hypolipidemic ($n = 4$ replicates)	8.2 ± 0.7	17.5 ± 1.8	12.2 ± 3.3	59.1 ± 3.4	97.0 ± 2.6	2.2 ± 0.8
normolipidemic ($n = 6$ replicates)	18.5 ± 2.1*	15.6 ± 3.2	11.1 ± 1.9*	53.5 ± 5.2	98.7 ± 2.8	11.6 ± 0.6*
hyperlipidemic ($n = 6$ replicates)	31.4 ± 3.3* **	21.0 ± 4.4	4.9 ± 1.0* **	37.5 ± 4.8* **	94.6 ± 4.8	14.6 ± 2.5*

[a] Data are expressed as mean ± standard; (*) $p < 0.05$ vs hypolipidemic patients; (**) p, 0.05 versus normolipidemic patients. [b] Percent of initial Hf·HCL concentration. [b] Percent of initial drug incubated; LPDP, lipoprotein-deficient plasma; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TRL, triglyceride-rich lipoproteins, which includes very-low-density lipoproteins and chylomirons; LTP I, lipid transfer protein I; k, fraction of labeled lipid transferred; t, time; plasma type, hypolipidemic (total cholesterol < 130 mg/dL and triglyceride < 100 mg/dL); normolipidemic (total cholesterol 130–200 mg/dL and triglyceride 100–200 mg/dL); hyperlipidemic (total cholesterol > 350 mg/dL and triglyceride > 280 mg/dL).

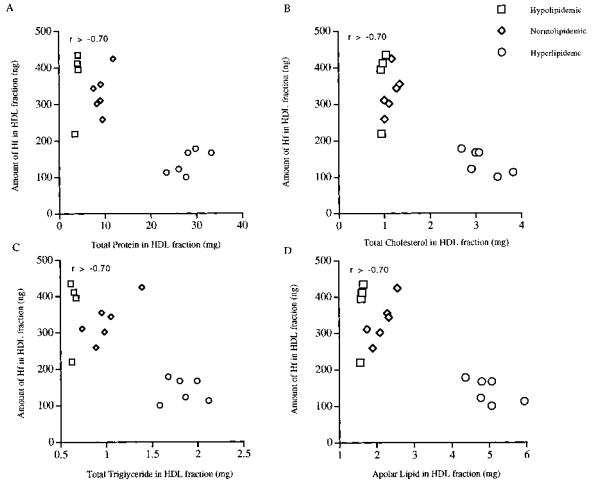

Figure 2—Distribution of Hf at 1000 ng/mL incubated for 60 min at 37 °C in hypolipidemic, normolipidemic, or hyperlipidemic plasma samples: (A) high-density lipoprotein (HDL) protein; (B) HDL cholesterol; (C) HDL TG; and (D) HDL apolar lipid (cholesteryl ester and triglyceride) content versus the amount of Hf within the HDL fraction.

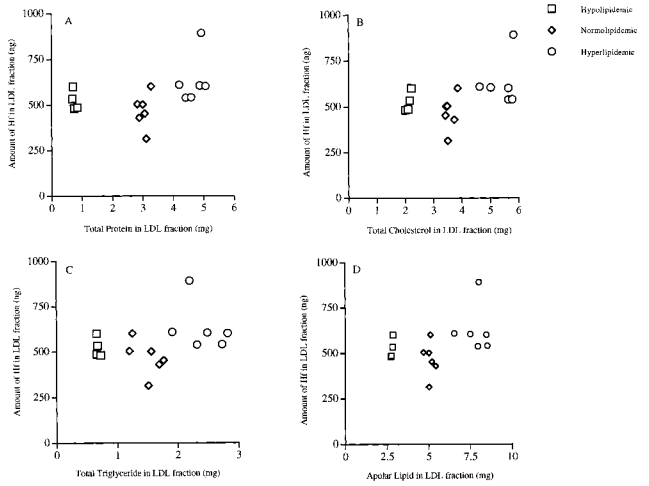

Figure 3—Distribution of Hf at 1000 ng/mL incubated for 60 min at 37 °C in hypolipidemic, normolipidemic, or hyperlipidemic plasma samples: (A) low-density lipoprotein (LDL) protein; (B) LDL cholesterol; (C) LDL TG; and (D) LDL apolar lipid (cholesteryl ester and triglyceride) content versus the amount of Hf within the LDL fraction.

When correlations between the amount of Hf recovered within the TRL, LDL, and HDL plasma fractions and the amount of cholesterol, triglyceride, and protein were calculated for all three patient plasma subgroups, the following relationships were observed. As TRL cholesterol, triglyceride, protein, and apolar lipid (cholesteryl esters and triglycerides) levels increased, the amount of Hf recovered in this fraction proportionally increased (Figure 1; $r > 0.70$). However, as HDL cholesterol, triglyceride, protein, and apolar lipid levels increased, the amount of Hf recovered in this fraction proportionally decreased (Figure 2; $r > 0.70$). Although no correlation between LDL lipid and protein levels and the amount of Hf recovered in this fraction was observed (Figure 3), a positive correlation between the proportion of apolar lipid and percentage of Hf recovered within each lipoprotein fraction was observed within all three plasma groups (Figure 4; $r > 0.70$).

We have further observed that although HDL cholesterol, triglyceride, and protein concentrations increased 3-fold, 3-fold, and 7-fold, respectively, from hypolipidemia through to hyperlipidemia (Table 1), the percent (Table 3)

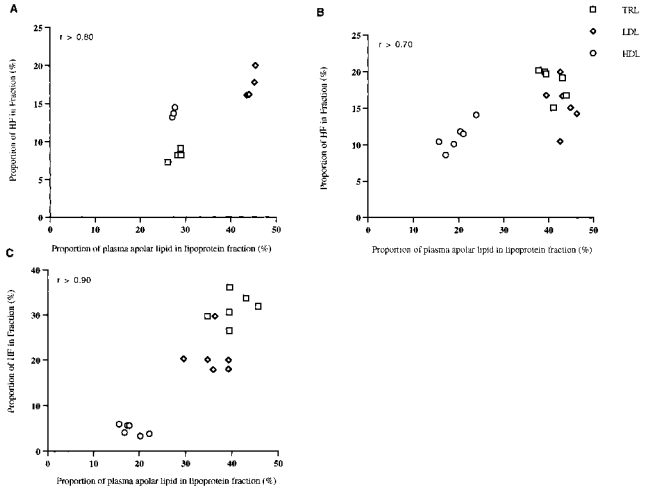

Figure 4—(A) Correlation between the percentage of Hf recovered in triglyceride-rich (TRL), low-density (LDL), and high-density lipoprotein (HDL) fractions in hypolipidemic human plasma and the proportional distribution of apolar lipids (cholesteryl esters and triglycerides) within the individual lipoprotein fractions. (B) Correlation between the percentage of Hf recovered in TRL, LDL, and HDL fractions in normolipidemic human plasma and the proportional distribution of apolar lipids (cholesteryl esters and triglycerides) within the individual lipoprotein fractions. (C) Correlation between the percentage of Hf recovered in TRL, LDL, and HDL fractions in hyperlipidemic human plasma and the proportional distribution of apolar lipids (cholesteryl esters and triglycerides) within the individual lipoprotein fractions.

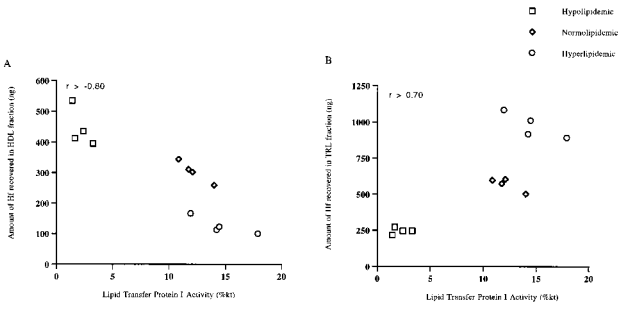

Figure 5—Lipid transfer protein I activity in hypolipidemic, normolipidemic, or hyperlipidemic plasma samples versus the amount of Hf within the (A) high-density lipoprotein (HDL) and (B) triglyceride-rich lipoprotein (TRL) fractions.

Table 4—Influence of LTP I on the Distribution of Halofantrine Hydrochloride (Hf·HCl) at 1000 ng/mL within Normolipidemic Human Plasma Following Incubation for 60 min at 37 °C (following incubation, plasma samples were assayed by high-pressure liquid chromatography for drug in each of the lipoprotein and lipoprotein-deficient plasma fractions)[a]

drug compound	TRL plasma fraction, %[b]	LDL plasma fraction, %	HDL plasma fraction, %	LPDP fraction, %	percent recovery
Hf·HCL	19.9 ± 2.7	20.8 ± 2.5	17.1 ± 1.1	42.1 ± 2.7	83.2 ± 4.2
Hf·HCL + LTP I	44.9 ± 2.7*	12.7 ± 2.0*	7.9 ± 1.2*	34.6 ± 1.9*	98.3 ± 8.9*

[a] Data are expressed as mean ± standard deviation ($n = 6$); (*) $p < 0.05$ vs Hf·HCl; 33 μL CM-LTP I (containing 0.67 μg of LTP I) added to 1 mL of plasma. [b] Percent of initial Hf·HCL concentration. [c] Percent of initial drug incubated; LPDP, lipoprotein-deficient plasma; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TRL, triglyceride-rich lipoproteins, which includes very low-density lipoproteins and chylomicrons; LTP I, lipid transfer protein I; normolipidemic human plasma had a total cholesterol of 161 mg/dL and endogenous LTP I concentration of 1.05 μg protein/mL.

and amount (Figure 2) of Hf recovered within the HDL fraction significantly decreased. However, in these same plasmas, TRL cholesterol, triglyceride, and protein concentrations increased 5.5-fold, 5-fold, and 10-fold, respectively, from hypolipidemia through to hyperlipidemia (Table 1). This resulted in a significant and proportional increase in the percent (Table 3) and amount (Figure 1) of Hf recovered within the TRL fraction. These findings suggest that Hf lipoprotein distribution may be regulated by mass plasma lipoprotein triglyceride concentrations and the number of TRL particles present within plasma. Thus, Hf moved from one lipoprotein class to another and its movement could be influenced by different disease states, such as malaria, and adjunct therapies, such as Intralipid infusion,[3] where lipoprotein composition and plasma concentrations are altered. Furthermore, not only are triglyceride mass and particle number important factors in determining Hf lipoprotein distribution, but to which lipoprotein subfraction changes in composition ocurr may also be important in determining to which component the drug binds. It further appears that TRL may be more effective at binding Hf than either HDL or LDL as you increase lipoprotein lipid-load and particle number.

Influence of Lipid Transfer Protein I (LTP I) on the Plasma Distribution of Halofantrine (Hf)—The LTP I activity within the plasma of these patients were determined by measuring the transfer rate of [^{3}H]CE from LDL to HDL. As the total plasma and lipoprotein lipid concentrations increased from hypolipidemia through to hyerlipidemia, LTP I activity increased from 2.2 ± 0.8% k_t to 14.6 ± 2.5% k_t (Table 3). Furthermore, as LTP I-mediated transfer of CE increased, the amount of Hf recovered in the HDL fraction decreased (Figure 5A), whereas the amount of Hf recovered in the TRL fraction increased (Figure 5B). In addition, a significantly lower percentage of Hf was recovered in the LDL, HDL, and LPDP fractions and a significantly greater percentage of Hf was recovered in the TRL fraction compared with controls when fasted normolipidemic plasma containing 1 μg protein/mL of LTP I was supplemented with 0.67 μg protein/mL of exogenous LTP I (Table 4).

Taken together, these studies demonstrate that as LTP I activity increases, the proportion of Hf associated with the TRL fraction increases ($r > 0.70$) and the proportion of Hf associated with HDL decreases ($r > 0.80$). Because LTP I is the protein that catalyzes the transfer exchange of apolar lipids CE from CE-rich lipoproteins (HDL and LDL) for TG from TG-rich lipoproteins (i.e., VLDL and chylomicrons), our findings suggest that Hf plasma distribution may be related to its lipoprotein apolar lipid content. This suggestion is further supported by the observation within hypo- ($r > 0.80$), normo- ($r = 0.70$), and hyperlipidemic ($r > 0.90$) plasma samples that a proportionally greater percentage of Hf was recovered in those lipoprotein fractions that contained a larger proportion of apolar lipid (Figure 4). These findings are in agreement with our previous studies in pre- and postprandial plasma samples from beagles and humans,[9] where it was concluded that the mechanism of Hf association with plasma lipoprotein was primarily by way of solubilization in the apolar lipid core.

Summary

In conclusion we have determined that Hf associates with lipoproteins upon entrance into the plasma component of the bloodstream and that the distribution of Hf among lipoproteins is defined by the relative levels of individual lipoproteins and the proportion of apolar lipid content within each lipoprotein fraction, and is influenced by LTP I. Furthermore, the amount of Hf recovered in the TRL and HDL fractions correlates to lipoprotein lipid and protein mass and LTP−I activity. The alterations in plasma lipoprotein concentrations, including decreases in HDL-cholesterol and elevations in TRL-triglyceride concentrations,[8] often exhibited by patients with malaria, raises the possibility of altered efficacy and/or toxicity of drugs, such as Hf, which associate with lipoproteins. Recently, Humberstone et al.[11] demonstrated that the IC_{50} of Hf was significantly increased when incubated in the presence of 10% post-prandial serum (which contains elevated TRL triglyceride serum concentrations) compared with normolipidemic serum in a continuous in vitro culture of *Plasmodium falciparum*. In addition, there is an apparent linkage between excessively high plasma Hf concentrations and cardiac toxicity in patients with or without a preexisting cardiopathy.[22] As higher plasma concentrations of Hf are typically observed after post-prandial administration, distribution of Hf into post-prandial lipoproteins (particularly TRL) may contribute to the cardiac side effects of Hf observed in these patients. Future studies are warranted to investigate the pharmacological implications of the predominant association of Hf with TRL when total plasma TG concentrations are elevated.

References and Notes

1. Davis, R. A.; Vance, J. E. Structure, assembly and secretion of lipoproteins. In *Biochemistry of lipids, lipoproteins and membranes;* Vance, D. E.; Vance, J. E., Eds.; Elsevier: New York, 1996; pp 473−493.
2. Wasan, K. M.; Vadiei, K.; Lopez-Berestein, G.; Luke, D. R. Pharmacokinetics, tissue distribution, and toxicity of free and liposomal amphotericin B. *J. Infect. Dis.* **1990**, *161*, 562−566.
3. Wasan, K. M.; Grossie, Jr., V. B.; Lopez-Berestein, G. Concentrations in serum and tissue distribution of free and liposomal amphotericin B in rats on continuous Intralipid infusion. *Antimicrob. Agents Chemother.* **1994**, *38*, 2224−2226.
4. Le Maire, M.; Tillement, J. P. Role of lipoproteins and erythrocytes in the in vitro binding and distribution of cyclosporin A in the blood. *J. Pharm. Pharmacol.* **1982**, *34*, 715−718.
5. Wasan, K. M.; Morton, R. E. Differences in lipoprotein concentration and composition modify the plasma distribution of free and liposomal annamycin. *Pharm. Res.* **1996**, *13*, 462−468.

6. Wasan, K. M.; Perez-Soler, P. Distribution of free and liposomal annamycin within human plasma is regulated by plasma triglyceride concentrations but not by lipid transfer protein. *J. Pharm. Sci.* **1995**, *84*, 1094−1100.
7. Wasan, K. M.; Pritchard, P. H.; Ramaswamy, M.; Wong, W.; Donnachie, E. M.; Brunner, L. J. Differences in lipoprotein lipid concentration and composition modify the plasma distribution of cyclosporine. *Pharm. Res.* **1997**, *14*, 1613−1620.
8. Cenni, B.; Meyer, J.; Brandt, R.; Betschart, B. The antimalarial drug halofantrine is bound mainly to low and high-density lipoproteins in human serum. *Br. J. Clin. Pharmacol.* **1995**, *39*, 519−526.
9. McIntosh, M. P.; Porter, C. J. H.; Wasan, K. M.; Ramaswamy, M.; Charman. W. N. Differences in the lipoprotein binding profile of halofantrine in fed and fasted human or beagle plasma are dictated by the respective masses of core apolar lipoprotein lipid. *J. Pharm. Sci*., in press.
10. Humberstone, A. J.; Porter, C. J. H.; Edwards, G.; Charman, W. N. Distribution of halofantrine between plasma lipoprotein fractions after IV administration to pre- and postprandial beagle dogs. *Pharm. Res.* **1995**, *12*, S-356 (abstract).
11. Humberstone, A. J.; Porter, C. J. H.; Edwards, G.; Charman, W. N. Effect of altered serum lipid concentrations on the IC_{50} of halofantrine against *Plasmodium falciparum*. *J. Pharm. Sci.* **1998**, *87*, 256−258.
12. Morton, R. E.; Zilversmit, D. B. Purification and characterization of lipid transfer protein(s) from human lipoprotein-deficient plasma. *J. Lipid Res.* **1982**, *23*, 1058−1067.
13. Wasan, K. M.; Morton, R. E.; Rosenblum, M. G.; Lopez-Berestein, G. Decreased toxicity of liposomal amphotericin B due to association of amphotericin B with high-density lipoproteins: Role of lipid transfer protein. *J. Pharm. Sci.* **1994**, *83*, 1006−1010.
14. Wasan, K. M.; Ramaswamy, M.; Wong, W.; Pritchard, P. H. Lipid transfer protein I facilitated transfer of cyclosporine from low- to high-density lipoproteins is only partially dependent on its cholesteryl ester transfer activity. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 509−605.
15. Havel, R. J.; Eder, H. A.; Bragdon, J. H. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **1955**, *34*, 1345−1353.
16. Ramaswamy, M.; Zhang, X.; Burt, H. M.; Wasan, K. M. The human plasma distribution of free paclitaxel and paclitaxel associated with diblock copolymers. *J. Pharm. Sci.* **1997**, *86*, 460−464.
17. Burstein, M.; Scholnick, H. R.; Morfin, R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **1970**, *11*, 583−595.
18. Wasan, K. M.; Ramaswamy, M.; Haley, J.; Dunn, B. P. Administration of long-term tamoxifen therapy modifies the plasma lipoprotein−lipid concentration and lipid transfer protein I activity in postmenopausal women with breast cancer. *J. Pharm. Sci.* **1997**, *86*, 876−879.
19. Pattnaik, N. M.; Zilversmit, D. B. Interaction of cholesteryl ester exchange protein with human plasma lipoproteins and phospholipid vesicles. *J. Biol. Chem*. **1979**, *254*, 2782−2786.
20. Humberstone, A. J.; Currie, G. J.; Porter, C. J. H.; Scanlon, M. J.; Charman, W. N. A simplified liquid chromatography assay for the quantitation of halofantrine and desbutylhalofantrine in plasma and identification of a degradation product of desbutylhalofantrine formed under alkaline conditions. *J. Pharm. Biomed. Anal.* **1995**, *13*, 265−272.
21. Karbwang, J.; Na Bangchang, K. Clinical pharmacokinetics of halofantrine. *Clin. Pharmacokinet.* **1994**, *27*, 104−119.
22. Monlun, E.; Le Metayer, P.; Szwandt, S.; Neau, D.; Longy Boursier, M.; Horton, J.; Le Bras, M. Cardiac complications of halofantrine: a prospective study of 20 patients. *Trans. Roy Soc. Trop. Med. Hyg.* **1995**, *89*, 430−433.

Acknowledgments

This work was supported in part by the University of British Columbia Development Fund and SmithKline Beecham Pharmaceuticals Inc. (UK). The authors thank Dr. John Horton for his interest and support of these studies and Dr. Andrew Humberstone for his initial discussions and feedback.

JS980353K