# Cyclosporine Transfer from Low- and High-Density Lipoproteins Is Partially Influenced by Lipid Transfer Protein I Triglyceride Transfer Activity

**Kishor M. Wasan,[1,4] Ramaswamy Subramanian,[1] Jenny Wen-Lin Chou,[1] Manisha Ramaswamy,[1] and P. Haydn Pritchard[3]**

*Received January 19, 1999; accepted March 18, 1999*

***Purpose.*** The purpose of this study was to determine if lipid transfer protein (LTP I) facilitated triglyceride (TG) transfer activity regulates the plasma lipoprotein distribution of cyclosporine (CSA).

***Methods.*** To assess the influence of drug concentration and incubation time on the plasma lipoprotein distribution of CSA, $^3$H-CSA (50 to 1000 ng/ml) was incubated in human plasma for 5 to 120 minutes at 37°C. To determine if LTP I facilitated TG transfer activity regulates the plasma lipoprotein distribution of CSA, $^3$H-Triolein (TG)- or $^3$H-CSA-enriched high-density lipoproteins (HDL) or low-density lipoproteins (LDL) were incubated in T150 buffer (50 mM Tris-HCl, 150 mM NaCl, 0.02% Sodium Azide, 0.01% Disodium EDTA), pH 7.4 which contained a $^3$H-Triolein (TG) or $^3$H-CSA-free lipoprotein counterpart ± exogenous LTP I (1.0 μg protein/ml) or in delipidated human plasma which contained 1.0 μg protein/ml of endogenous LTP I for 90 minutes at 37°C. These experiments were repeated in the presence of a monoclonal antibody TP1 (15 μg protein/ml) directed against LTP I.

***Results.*** No differences in CSA lipoprotein distribution were observed following incubation of the drug at varying concentrations and incubation times in human plasma. The percent transfer of TG from HDL to LDL and LDL to HDL was greater in T150 buffer than in human plasma. However, the percent transfer of CSA from only LDL to HDL was greater in T150 buffer than in human plasma. Furthermore, undetectable $^3$H-CSA transfer from HDL to LDL in T150 buffer containing purified LTP I was observed. In addition, when the percent transfer of TG and CSA were determined in the presence of TP1, the percent transfer of TG and CSA from only LDL to HDL were significantly decreased in T150 buffer and human plasma compared to controls.

***Conclusions.*** These findings suggest that the transfer of CSA between different lipoprotein particles is only partially influenced by LTP I facilitated TG transfer activity.

**KEY WORDS:** lipid transfer protein I; cyclosporine; lipoproteins; triglycerides.

## INTRODUCTION

Lipid transfer protein I (LTP I), often referred to as cholesteryl ester transfer protein (1), is a glycoprotein with a molecular weight of 74,000 that has been shown to facilitate the transfer of cholesteryl esters (CE), triglyceride (TG) and phospholipids between different plasma lipoprotein particles (2–4). Although other investigators have demonstrated the presence of distinct TG and phospholipid transfer proteins (5,6) which lack the capacity to transfer neutral lipids (1,7), LTP I remains the best characterized lipid transfer protein in plasma.

Recently, it has been hypothesized that an increase in LTP I concentration and activity may facilitate the movement of lipophilic drugs, including the immunosuppressant cyclosporine (CSA), among different lipoprotein classes (8,9). We have previously demonstrated that CSA predominantly associates with high-density lipoproteins (HDL) and low-density lipoproteins (LDL) upon incubation in plasma (10). However, when human plasma was supplemented with exogenous LTP I, CSA redistributes from LDL to HDL (8). Furthermore, we observed that LTP I facilitated transfer of CSA between HDL and LDL was only partially dependent on its CE transfer activity (8). These observations in part suggest that changes in LTP I concentration may regulate the distribution of CSA among the different lipoprotein particles within human plasma.

Our laboratory has recently reported that changes in the total and plasma lipoprotein lipid concentration and composition influence the lipoprotein association of CSA (10). Specifically, as very low-density lipoprotein (VLDL) and LDL cholesterol and triglyceride concentrations increased, the percent of CSA recovered within these fractions increased. Furthermore, the percent of CSA recovered within the HDL fraction significantly decreased as HDL triglyceride concentrations increased.

One of the proposed biological consequences of CSA association to lipoproteins is the decrease in the drug's pharmacological effect. Several investigators have reported decreased pharmacological effects of CSA with hyperlipidemia (particularly in hypertriglyceridemia) (11,12), and increased toxic effects of CSA with hypolipidemia (particularly hypocholesterolemia) (13). Lemaire and coworkers have suggested that the drug's availability to tissue and hence, its pharmacological (or toxic) effects may depend upon which lipoprotein the drug is bound (14). They have observed enhanced antiproliferative effect of CSA when it was bound to LDL, which was not evident, when the drug was bound to either VLDL or HDL

[1] Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada.

[2] This work was supported with a grant from the Medical Research Council of Canada (Grant #MT-14484). Jenny Wen-Lin Chou was supported by a summer student research fellowship from the Heart & Stroke Foundation of British Columbia and Yukon.

[3] Department of Pathology and Laboratory Medicine, Faculty of Medicine, The University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada.

[4] To whom correspondence should be addressed. (e-mail: kwasan@unixg.ubc.ca)

**ABBREVIATIONS:** LTP I, lipid transfer protein I; CE, cholesteryl ester; TG, triglyceride; AmpB, amphotericin B; HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very-low-density lipoproteins; CSA, cyclosporine; IMP, imipramine; T 150 Buffer, 50 mM Tris-HCl, 150 mM NaCl, 0.02% sodium azide, 0.01% disodium EDTA, pH 7.4; TP1, 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.



0724-8741/99/0700-1067$16.00/0 

(14,15). Furthermore, transplantation patients who are administered CSA, exhibit plasma dyslipidemias (i.e., lipid disturbances) including hypocholesterolemia and hypertriglyceridemia (16,17) that have elevated LTP I levels (18). Thus, determining if LTP I facilitate the binding of CSA to certain lipoproteins may help to explain differences in CSA's pharmacological behavior following administration to hypocholesterolemic (13) and/or hypertriglyceridemic patients (11,12).

The objectives of this study were to determine if LTP I facilitated transfer of TG regulates the plasma lipoprotein distribution of CSA. Since LTP I facilitates TG, as well as CE, movement between HDL and LDL and increases in TG lipoprotein concentrations modifies the lipoprotein distribution of CSA, we hypothesized that the transfer of CSA between HDL and LDL may be a result of co-transport of CSA and TG by LTP I.

## MATERIALS AND METHODS

### Chemicals and Plasma

Radiolabeled CSA ([*mebmt*-β-$^3$H] Cyclosporin A; Specific Activity, 7.39 mCi/mg), IMP ([$^3$H]Imipramine; Specific Activity, 78.0 mCi/mg and radiolabeled triolein (Glycerol tri [9,10 (n)-$^3$H] oleate; specific activity, 23.7 mCi/mg; serving as a negative control) were purchased from Amersham Life Science (Mississauga, Ontario, Canada). Sodium bromide was purchased from Sigma Chemical Company (St. Louis, MO). Normolipidemic fasted human plasma was obtained from the Vancouver Red Cross (Vancouver, British Columbia). Ten microliters of 0.4M ethylenediaminetetraacetic acid pH 7.1 (EDTA, Sigma Chemical Company) was added to 1.0 ml of whole blood. For all CSA and IMP plasma distribution studies, $^3$H-CSA and $^3$H-IMP were dissolved in a 100% ethanol solution. However, the volume of ethanol used did not modify lipoprotein composition or LTP I activity (data not shown).

### Lipoprotein Separation

In the preparation of lipoproteins containing $^3$H-CSA and $^3$H-Triolein the plasma was separated into its HDL and LDL fractions by sequential ultracentrifugation (19,20). Briefly, human plasma (3.0 ml) samples were placed in centrifuge tubes and their solvent densities adjusted to 1.006 g/ml by sodium bromide. Following centrifugation (L8-80M; Beckman Canada) at 50,000 rpm for 18 hours at 4°C the VLDL-rich and VLDL-deficient plasma fractions were recovered. The VLDL-deficient plasma fraction was readjusted to a density of 1.063 g/ml and respun at 50,000 rpm for 18 hours at 4°C to separate the LDL-rich and VLDL/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 LPDP fractions. For all plasma lipoprotein distribution studies of CSA and IMP the plasma was separated into its HDL, LDL, VLDL and lipoprotein-deficient plasma (LPDP) fractions by step-gradient ultracentrifugation as previously described (8).

Sequential ultracentrifugation was used to isolate and purify HDL and LDL because of the large quantities of lipoprotein that can be obtained with minimal contamination (21). However, our laboratory has recently reported that step-gradient ultracentrifugation is the preferred method for drug distribution studies (21). In addition, previous studies have shown that CSA plasma lipoprotein distribution was not altered when using different lipoprotein separation techniques (i.e., step-gradient ultracentrifugation versus affinity chromatography versus fast protein liquid chromatography) (10). Thus all drug incubation studies used step-gradient ultracentrifugation to separate lipoprotein and lipoprotein-deficient plasma fractions.

### Isolation and Purification of Lipid Transfer Protein (LTP I)

LTP I was purified from human lipoprotein-deficient plasma as has been previously described (2). Briefly, citrated human plasma was made lipoprotein-deficient by the dextran-$MnCl_2$ procedure of Burstein and co-workers (22). LTP I was then partially purified by sequential chromatography on phenyl-Sepharose and carboxy-methylcellulose gel (CMC-52, Whatman Inc., Chifton, NJ). Purified LTP I (2.0-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 CMC fraction of LTP I was used in all experiments. Lipid transfer protein I-free CMC solution does not elicit any lipid or drug transfer activity (data not shown).

### Radiolabeling of Plasma Lipoproteins

Human HDL and LDL were labeled by the lipid dispersion technique as previously described (2,3). Briefly, human plasma was incubated with a lipid dispersion containing egg phosphatidylcholine (PC) and $^3$H-Triolein (210 ng/ml) or $^3$H-CSA (1000 ng/ml) at 37°C for 20–24 h in the presence of LTP I. The plasma was then separated into its HDL and LDL fractions by ultracentrifugation as previously described and further purified by dialyzing against phosphate buffered saline (PBS) solution (4 liters) for 18 hours at 4°C. The molecular weight cutoff of the dialysis tubing used was 1,000. Following dialysis these lipoprotein fractions were filtered through a 0.2-micron filter. The dialysis and filtration steps were performed to remove any radiolabeled triolein or CSA, which has not been incorporated into the core of HDL and LDL.

### Lipid and Drug Transfer Assays

Lipid (TG) and drug (CSA) transfers were performed within the T150 buffer and lipoprotein-deficient plasma using a modified method as has been previously described (2,8,23,24). Typically, 10 μg (total TG) 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 T150 buffer or 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 minutes (time was determined from a time response curve [data not shown]) at 37°C. Lipid and drug transfer between donor and acceptor lipoprotein is then quantitated by scintillation counting. The fraction of lipids and drug transferred (kt) is calculated as described by Pattnaik and Zilversmit (24):

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

where $D_0$ and $A_t$ are the radioactivities of the donor at time 0 and 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 and drug transfer is an exchange process. In order to minimize calculation errors due to mass transfer, all values will be determined from assays in which the extent of radio label transfer is small (<15%).

### Quantification of Cyclosporine (CSA), Imipramine (IMP) and Plasma Lipids

HDL, LDL, VLDL, and LPDP fractions were analyzed for $^3$H-CSA and $^3$H-IMP against external standard calibration curves (corrected for quenching and luminescence) using radioactivity. Enzymatic assay kits from Sigma Diagnostics (St. Louis, Mo.) were used to determine total and lipoprotein cholesterol and TG concentrations.

### Experimental Design

To assess if changes in drug concentration and incubation time modify the plasma lipoprotein of CSA, $^3$H-CSA at 50, 100, 250, 500 and 1000 ng/ml was incubated [peak concentrations of 1000 ng/ml were observed following intravenous administration (25)] in normolipidemic human plasma (total cholesterol = 100–200 mg/dl; total triglyceride = 100–200 mg/dl) for 1, 5, 30, 60, 90 and 120 minutes at 37°C (Fig. 1). Furthermore, $^3$H-IMP at a physiologic concentration of 150-ng/ml (26) was incubated in human plasma for 5 through 90 minutes at 37°C (Fig. 2). IMP was chosen as a negative control, since it is an example of a relatively hydrophobic compound [log P = 4.6] (26) with low lipoprotein binding (31).

To establish that HDL and LDL have the ability to sequester CSA and TG within their hydrophobic lipid core, radiolabeled CSA and TG were incubated in human plasma and the amount of radiolabeled CSA and TG incorporated into HDL and LDL were determined (Table 1).

To investigate the hypotheses that the transfer of CSA between lipoproteins is regulated by LTP I facilitated TG transfer activity, the experimental conditions to be used for LTP I studies were established (Tables 1 & 2, Fig. 1). Furthermore, two strategies that involved the supplementation and inhibition of LTP I were used to test the aforementioned hypotheses.

The first strategy was to incubate $^3$H-CSA -enriched HDL or LDL in 50 mM Tris-HCl, 150 mM NaCl, 0.02% sodium azide, 0.01% disodium ETDA (T150 buffer), pH 7.4 which contain a drug-free lipoprotein counterpart in the presence or absence of a purified source of LTP I (1.0 μg protein/ml) (Tables 3 & 4). Endogenous LTP I concentration within normolipidemic human plasma is usually 1–2 μg protein/ml (2,3). In a further experiment LTP I was co-incubated with TP1 (15 μg protein/ml) a monoclonal antibody directed against LTP I (27) (Tables 2 & 4). These experiments were designed to further confirm if the movement of CSA between lipoprotein particles was partially facilitated by LTP I and/or a result of non-facilitated drug transfer rather than the influence of other plasma components (i.e., phospholipid transfer proteins).

A second strategy was to incorporate CSA into HDL and LDL, re-isolate these CSA enriched lipoprotein fractions and then incubate these lipoprotein particles in lipoprotein-deficient human plasma in the presence of a drug-free lipoprotein counterpart (e.g., $^3$H-CSA-HDL and CSA-free LDL) (Tables 3 & 4). The human plasma, which served as the source of LTP I in this experiment, contained a LTP I concentration of 1.0 μg protein/ml as determined by ELISA (data not shown). To confirm that the transfer of CSA is due to LTP I and not other endogenous plasma factors, TP1 (15 μg protein/ml respectively; as determined by ELISA) (data not shown) was co-incubated with CSA-enriched and -free lipoprotein particles in plasma (Table 4). To assure that the antibody significantly inhibits LTP I facilitated TG transfer activity, TG transfer from LDL to HDL in the presence and absence of TP1 (Table 2) was determined. These experiments were designed to directly measure the potential role of LTP I in mediating drug transfer versus the ability of the drug molecules to spontaneously transfer among lipoprotein classes within human plasma.

For all the aforementioned experiments incubations were carried out for 90 minutes at 37°C. Following each incubation, the plasma and T150 buffer samples were separated into their

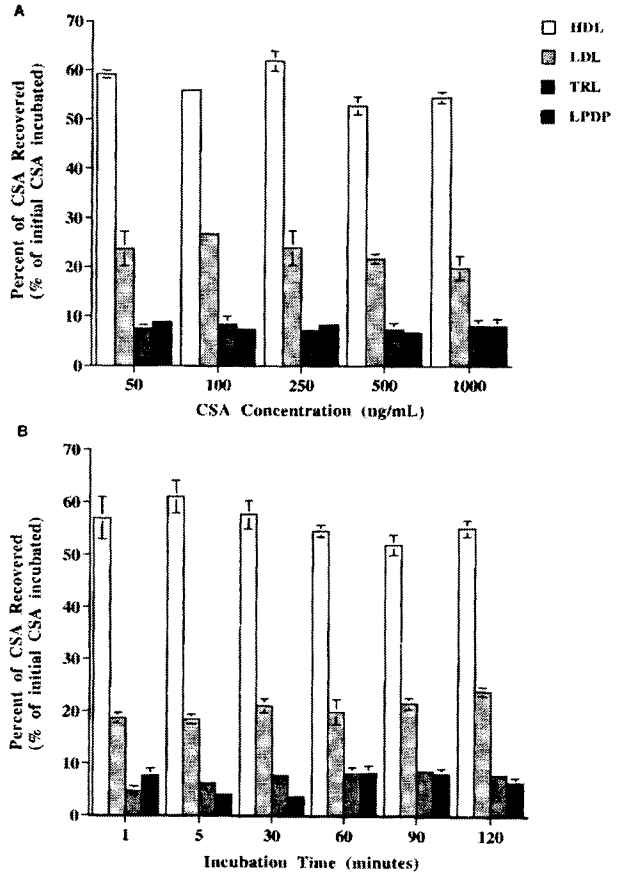


**Fig. 1.** Percent recovery of Cyclosporine (CSA) in the high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride-rich lipoprotein (which contains very low-density lipoproteins and chylomicrons) (TRL) and lipoprotein-deficient (which contains albumin and α-1 glycoproteins) (LPDP) fractions within human plasma. A. Cyclosporine from 50 to 1000 ng/ml was incubated in human plasma for 60 minutes at 37°C or B. Cyclosporine at 1000 ng/ml was incubation in human plasma for 1 to 120 minutes at 37°C. Following incubation the plasma was separated into its HDL, LDL, TRL, and LPDP fractions and the percentage of CSA recovered in each of these fractions was determined by radioactivity. Data was expressed as mean +/− standard deviation (n = 6).

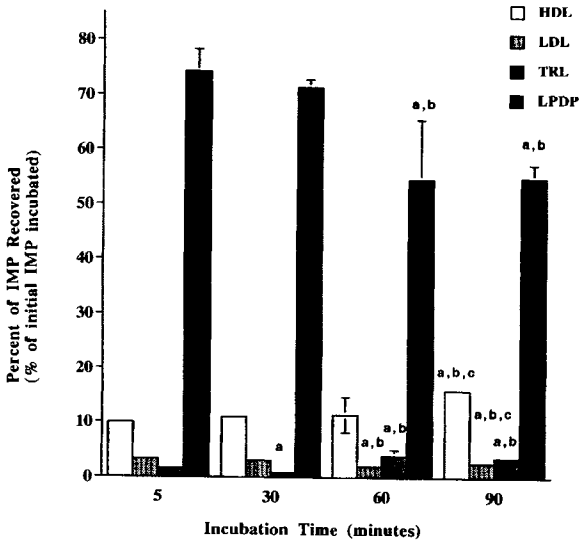


**Fig. 2.** Percent recovery of Imipramine (IMP) in the high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride-rich lipoprotein (which contains very low-density lipoproteins and chylomicrons) (TRL) and lipoprotein-deficient (which contains albumin and α-1 glycoproteins) (LPDP) fractions within human plasma. Imipramine at 150 ng/ml was incubation in human plasma for 5 to 90 minutes at 37°C. Following incubation the plasma was separated into its HDL, LDL, TRL, and LPDP fractions and the percentage of IMP recovered in each of these fractions was determined by radioactivity. Data was expressed as mean ± standard deviation (n = 6). [a] $p < 0.05$ vs. 5 minutes incubation time, [b] $p < 0.05$ vs. 30 minutes incubation time, [c] $p < 0.05$ vs. 60 minutes incubation time.

individual lipoprotein constituents by step-gradient ultracentrifugation and assayed for CSA by radioactivity.

### Statistical Analysis

Differences in drug distribution within plasma lipoproteins and differences in LTP I mediated TG and CSA transfer activity in the presence of different treatment groups were analyzed by one way analysis of variance (PCANOVA; Human Systems Dynamics). Critical differences were assessed by Neuman-Keuls posthoc tests. Differences were considered significant if $p < 0.05$. All data are expressed as mean ± standard deviation.

**Table 1.** Ability of HDL and LDL to Bind $^3$H-Triolein[a] and $^3$H-CSA[b] at 37°C After an Incubation of 24 Hours

| Lipoprotein Fraction | $^3$H-Triolein (ng $^3$H-Triolein/μg TG) | $^3$H-CSA (ng $^3$H-CSA/μg TG) |
|---|---|---|
| HDL | 0.25 ± 0.01 | 0.085 ± 0.002 |
| LDL | 0.19 ± 0.01* | 0.062 ± 0.006** |

Abbreviations: $^3$H, tritium; TG, triglyceride; CSA, cyclosporine; HDL, high-density lipoproteins; LDL, low-density lipoproteins. Data are presented as mean ± standard deviation (n = 3).
[a] 0.21 μg of $^3$H-Triolein was incubated in 1 mL of human plasma.
[b] 1 μg of $^3$H-cyclosporine was incubated in 1 mL of human plasma with endogenous lipid transfer protein concentration of 1.0 μg protein/mL.
* P < 0.05 vs HDL.
** P < 0.05 vs HDL.

**Table 2.** Ability of LTP I Monoclonal Antibody (TP1) in Various Concentrations to Influence the Transfer of Radiolabeled TG from LDL to HDL at 37°C After an Incubation of 90 Minutes

| TG Transfer with Monoclonal Antibody | | | |
|---|---|---|---|
| Concentration (μg TP1/mL plasma) | Percent Transfer (%) | Percent Control (%) | Percent Inhibition (%) |
| 0 | 11.1 ± 0.7 | 100 | 0 |
| 5 | 7.1 ± 0.9* | 64.0 | 36.0 |
| 10 | 6.8 ± 1.0* | 61.3 | 38.7 |
| 15 | 6.1 ± 1.7* | 55.0 | 45.0 |
| 30 | 5.9 ± 1.2* | 53.0 | 47.0 |

Abbreviations: LTP I, lipid transfer protein I; TG, triglyceride; LDL, low-density lipoproteins; HDL, high-density lipoproteins. Data are presented as mean ±/− standard deviation (n = 5).
* $p < 0.05$ vs. "0" Concentration.

## RESULTS

### Influence of Drug Concentration and Incubation Time on the Plasma Lipoprotein Distribution of Cyclosporine (CSA) and Imipramine (IMP)

*Cyclosporine*

To assess the influence of drug concentration and incubation time on the plasma lipoprotein distribution of CSA, $^3$H-CSA (50–1000 ng/ml) was incubated in human plasma for 1, 5, 30, 60, 90 and 120 minutes at 37°C. As reported in Fig. 1A and 1B, the majority of CSA was recovered within the HDL regardless of drug concentration and incubation time. To determine the ability of HDL and LDL to bind CSA, $^3$H-CSA was incubated in human plasma for 24 hours at 37°C. HDL bound 0.085 ng of $^3$H-CSA per μg of HDL TG and LDL bound 0.062 ng of $^3$H-CSA per μg LDL TG (Table 1).

**Table 3.** Triglyceride and Cyclosporine Percent Transfer From HDL to LDL and LDL to HDL, Following the Incubation for 90 Minutes at 37°C in T150 Buffer Which has Been Supplemented with Purified LTP I (1.0 μg Protein/ml) [Purified LTP I] or Delipidated Human Plasma Which Contains 1.0 μg Protein/ml of LTP I [Plasma LTP I]

| Treatment Groups | Net TG transfer (% kt) | Net CSA transfer (% kt) |
|---|---|---|
| HDL to LDL | | |
| Plasma LTP I | 18.6 ± 2.1 | 16.8 ± 3.2 |
| Purified LTP I | 32.4 ± 1.7* | ND |
| LDL to HDL | | |
| Plasma LTP I | 15.6 ± 1.8 | 15.8 ± 1.0 |
| Purified LTP I | 27.4 ± 0.4* | 25.6 ± 0.8** |

Abbreviations: TG, triglyceride; CSA, cyclosporine; HDL, high-density lipoproteins; LDL, low-density lipoproteins; T150 buffer, phosphate buffered saline which contains 50 mM Tris-HCl, 150 mM NaCl, 0.02% sodium azide, 0.01% disodium EDTA, pH 7.4; LTP I, lipid transfer protein I; k, the fraction of label transferred per unit time; t, time; ND, non detectable. Data was expressed as mean ± standard deviation (n = 4). * $p < 0.05$ vs. TG percent transfer with plasma LTP I; ** $p < 0.05$ vs. CSA percent transfer with plasma LTP I.

**Table 4.** Triglyceride and Cyclosporine Percent Transfer from LDL to HDL, in the Presence or Absence of a Monoclonal Antibody (TP1) Directed Against Lipid Transfer Protein I, Following the Incubation of Radiolabeled TG- and CSA-Enriched LDL with Cold HDL (at 10 μg Lipoprotein TG) for 90 Minutes at 37°C in T150 Buffer which has been Supplemented with LTP I (1.0 μg Protein/ml) or Delipidated Human Plasma which Contains 1.0 μg Protein/ml of LTP I

| Treatment Groups | Net TG transfer (% kt) | Net CSA transfer (% kt) |
|---|---|---|
| T150 Buffer | | |
| Without TP1 | 27.4 ± 0.2 | 25.6 ± 0.8 |
| With TP1 | 0.5 ± 0.4* | 18.8 ± 2.5** |
| Human Plasma | | |
| Without TP1 | 9.2 ± 0.6 | 15.1 ± 1.9 |
| With TP1 | 1.2 ± 0.9* | 9.0 ± 2.1** |

Abbreviations: TG, triglyceride; CSA, cyclosporine; HDL, high-density lipoproteins; LDL, low-density lipoproteins; TP1, monoclonal antibody directed against LTP I; T150 buffer, phosphate buffered saline which contains 50 mM Tris-HCl, 150 mM NaCl, 0.02% sodium azide, 0.01% disodium EDTA, pH 7.4; LTP I, lipid transfer protein I; k, the fraction of label transferred per unit time; t, time; ND, non detectable. Data was expressed as mean ± standard deviation (n = 5). * $p < 0.05$ vs. TG percent transfer without TP1; ** $p < 0.05$ vs. CSA percent transfer without TP1.

Since no differences in CSA lipoprotein distribution were observed following incubation of the drug at varying concentrations and incubation times in human plasma, a concentration of 1000 ng/ml at an incubation time of 90 minutes was chosen for all further incubation experiments.

*Imipramine*

To confirm that density gradient ultracentrifugation does not alter the plasma distribution of different hydrophobic compounds, $^3$H-IMP (150 ng/ml) was incubated in human plasma for 5 through 90 minutes at 37°C. IMP a hydrophobic compound with a log P of 4.6 (26) was chosen because previous findings reported that the majority of the drug is recovered in the LPDP fraction and is predominantly bound to albumin and α-1 glycoprotein (31). As reported in Fig. 2, the majority of IMP was recovered within the LPDP fraction (which contains predominantly albumin and α-1 glycoprotein) regardless of drug incubation time. These findings are similar to what has been reported by others (31).

### Triglyceride (TG) and Cyclosporine (CSA) Transfer Between High- (HDL) and Low- (LDL) Density Lipoproteins

To determine the ability of LTP I to promote the transfer of TG and CSA from HDL to LDL, radiolabeled TG- or CSA-enriched HDL and radiolabeled TG- or CSA-free LDL particles were incubated in T150 buffer [which contained 1.0 μg protein/ml of exogenous LTP I as determined by ELISA (data not shown)] or in LPDP [which contained 1.0 μg protein/ml of endogenous LTP I as determined by ELISA (data not shown)] for 90 minutes at 37°C. The percent transfer of TG and CSA from HDL to LDL was greater in T150 buffer containing purified LTP I than in human plasma containing LTP I (Table 3). Furthermore, undetectable $^3$H-CSA transfer occurred from HDL to LDL in T150 buffer containing purified LTP I (Table 3) and the presence of TP1 did not alter CSA transfer from HDL to LDL in human plasma (data not shown). Thus all further CSA and TG transfer experiments using TP1 were done only from LDL to HDL.

### Triglyceride (TG) and Cyclosporine (CSA) Transfer from Low- (LDL) and High- (HDL) Density Lipoproteins in the Presence of TP1

To determine the ability of LTP I to promote the transfer of TG and CSA from LDL to HDL, radiolabeled TG- or CSA-enriched LDL and radiolabeled TG- or CSA-free HDL particles were incubated in T150 buffer (which contained 1.0 μg protein/ml of exogenous LTP I) or in LPDP (which contained 1.0 μg protein/ml of endogenous LTP I) for 90 minutes at 37°C. The percent transfer of TG and CSA from LDL to HDL were significantly greater in T150 buffer containing purified LTP I than in human plasma containing LTP I (Tables 3 & 4). However, when the percent transfer of TG and CSA were determined in the presence of TP1, the percent transfer of TG and CSA from LDL to HDL were significantly decreased in T150 buffer and human plasma compared to controls (Table 4).

## DISCUSSION

The objective of this study was to determine the influence of LTP I on the plasma lipoprotein distribution of CSA. Our data suggests that LTP I appears to have a direct role in the distribution of CSA among plasma lipoproteins. This is similar to our observations with AmpB (23). However, unlike AmpB, the transfer of CSA between HDL and LDL appears to be only partially dependent on LTP I-facilitated transfer of CE (8) and TG (Table 4).

We have previously demonstrated that the distribution of AmpB among HDL and LDL following incubation in human plasma is facilitated by LTP I. However, once AmpB was incorporated into 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 (23). We concluded from these studies that since AmpB interacts with free cholesterol and CE upon incubation in plasma (28, 29), 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.

In the case of CSA we previously reported that increases in LTP I concentration resulted in an increased percentage of CSA recovered in the HDL/LPDP fraction during short-term incubations (10). Furthermore, as reported in tables 3 and 4, we observed that the transfer of CSA between HDL and LDL appears to be partially facilitated through LTP I TG transfer activity. As LTP I is the protein which catalyzes the transfer exchange of CE from CE-rich lipoproteins (HDL and LDL) for TG from TG-rich lipoproteins (VLDL), these findings suggest that CSA plasma distribution could be partially explained by its lipoprotein TG content.

In experiments that were designed to directly measure the potential role of LTP I to facilitate CSA transfer based on HDL

and LDL TG content, LTP I-mediated percent transfer of TG from LDL to HDL in human plasma was significantly different from that of CSA (Table 4). The differences in the percent transfer of TG versus CSA may be attributed to an ability of LTP I to transfer lipid and drug separately. Furthermore, differences could be attributed to the ability of HDL and LDL particles to accumulate a higher amount of TG than CSA (e.g., HDL sequesters approximately 2.94 ng TG/ng CSA; LDL sequesters approximately 3.06 ng CE/ng CSA). Our findings further suggest that HDL particles bind CSA more effectively than LDL particles (Table 1).

Sgoutas and coworkers have proposed that the nature of CSA's association with HDL and LDL particles appears to be non-specific and of low affinity and high capacity suggesting that CSA is physically dissolved within the lipoprotein-lipid component (30). Furthermore, since CSA appears to be only partially recognized by LTP I as an endogenous lipid compound, LTP I's ability to transfer CSA between HDL and LDL is only part of the story. This is supported by evidence from our present work which demonstrates that while the percent transfer of CSA from LDL to HDL is only partially inhibited in T150 buffer containing purified LTP I and human plasma containing LTP I when in the presence of TP1, TG transfer is almost completely inhibited (Table 4). These findings suggest two possibilities, a) the spontaneous transfer of CSA and/or b) the facilitated transfer of CSA by other endogenous plasma factors (i.e., phospholipid transfer proteins). However, the percent transfer of CSA from HDL to LDL is undetectable in T150 buffer containing purified LTP I, while TG transfer is significant (Table 3). In addition, the percent transfer of CSA from HDL to LDL in human plasma containing LTP I is not inhibited in the presence of TPI (data not shown). Taken together, these findings further support the notion that the transfer of CSA from HDL to LDL is not LTP I-mediated and may be due to spontaneous and/or facilitated transfer by other endogenous plasma factors. Furthermore, these results suggest that LTP I may only be partially responsible for the greater capacity of HDL than LDL to accept CSA. Different physical-chemical characteristics of HDLs including lipid composition and overall particle charge may possibly explain CSA's preference to bind HDL. Studies that investigate these characteristics are currently being completed in our laboratory.

In conclusion we have determined that the distribution of CSA among lipoproteins is partially influenced by LTP I. Since many bone marrow transplantation patients exhibit lipid disturbances, including hypocholesterolemia and hypertriglyceridemia, these results may provide an explanation for the unpredictable and inconsistent pharmacokinetics and pharmacodynamics of CSA following administration. Future studies will investigate the pharmacological implications of CSA's predominant association with plasma lipoproteins.

## ACKNOWLEDGMENTS

The authors would like to thank Dr. Ross Milne for the generous donation of TP1 and Dr. Richard E. Morton for providing the purified LTP I for these studies.

## REFERENCES

1. A. R. Tall, E. Abreau, and J. Shuman. Separation of a plasma phospholipid transfer protein from cholesterol ester/phospholipid exchange protein. *J. Biol. Chem.* **258**:2174–2178 (1983).
2. R. E. Morton and D. B. Zilversmit. Purification and characterization of lipid transfer protein(s) from human lipoprotein-deficient plasma. *J. Lipid Res.* **23**:1058–1067 (1982).
3. R. E. Morton and D. B. Zilversmit. Inter-relationships of lipid transferred by the lipid transfer protein isolated from human lipoprotein-deficient plasma. *J. Biol. Chem.* **258**:11751–11756 (1983).
4. R. E. Morton. Interaction of lipid transfer protein with plasma lipoproteins and cell membranes. *Experimentia* **46**:552–560 (1990).
5. O. V. Rajaram, G. H. White, and P. J. Barter. Partial purification and characterization of a triacylglycerol-transfer protein from rabbit serum. *Biochim. Biophys. Acta.* **617**:383–390 (1980).
6. A. S. Jarnagin, W. Kohr, and C. Fielding. Isolation and specificity of a Mr 74,000 cholesteryl ester transfer protein from human plasma. *Proc. Natl. Acad. Sci. USA* **84**:1854–1860 (1987).
7. J. J. Albers, J. H. Tollefson, C-H Chen, and A. Steinmetz. Isolation and characterization of human lipid transfer proteins. *Arterioscle-rosis* **4**:49–58 (1984).
8. K. M. Wasan, M. Ramaswamy, W. Wong, and P. H. Pritchard. 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.* **284**:599–605 (1998).
9. 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).
10. K. M. Wasan, P. H. Pritchard, M. Ramaswamy, W. Wong, E. M. Donnachie, and L. J. Brunner. Differences in lipoprotein lipid concentration and composition modify the plasma distribution of cyclosporine. *Pharm. Res.* **14**:1613–1620 (1997).
11. J. Nemunaitis, H. J. Deeg, and G. C. Yee. High cyclosporin levels after bone marrow transplantation associated with hypertriglyceridemia. *Lancet* **i**:744–745 (1986).
12. N. De Kippel, J. Sennesael, J. Lamote, G. Ebinger, and J. De Keyser. Cyclosporin leukoencephalopathy induced by intravenous lipid solution. *Lancet* **339**:1114–1115 (1992).
13. P. C. de Groen, A. J. Aksamit, J. Rakela, G. S. Forbes, and R. A. F. From. Central nervous system toxicity after liver transplantation. *N. Eng. J. Med.* **317**:861–866 (1987).
14. M. Lemaire, W. M. Pardridge, and G. Chaudhuri. Influence of blood components on the tissue uptake indices of cyclosporin in rats. *J. Pharmacol. Exp. Ther.* **244**:740–743 (1988).
15. W. M. Pardridge. Carrier-mediated transport of thyroid hormones through the rat blood-brain barrier. Primary role of albumin-bound hormone. *J. Clin. Invest.* **64**:145–154 (1979).
16. A. M. Gardier, D. Mathe, X. Guedeney, J. Barre, C. Benvenutti, N. Navarro, L. Vernillet, D. Loisance, J. P. Cachera, B. Jacotot, and J. P. Tillement. Effects of plasma lipid levels on blood distribution and pharmacokinetics of cyclosporin A. *Ther. Drug Monit.* **15**:274–280 (1993).
17. M. Arnadottir, H. Thysell, and P. Nilsson-Ehle. Lipoprotein levels and post-heparin lipase activities in kidney transplant recipients: Ciclosporin-versus non-ciclosporin-treated patients. *Am. J. Kidney Dis.* **17**:700–717 (1991).
18. P. Moulin, G. B. Appel, H. N. Ginsberg, and A. R. Tall. Increased concentration of plasma cholesteryl ester transfer protein in nephrotic syndrome: role in dyslipidemia. *J. Lipid Res.* **33**:1817–1822 (1992).
19. M. Ramaswamy, X. Zhang, H. M. Burt, and K. M. Wasan. The human plasma distribution of free paclitaxel and paclitaxel associated with diblock copolymers. *J. Pharm. Sci.* **86**:460–464 (1997).
20. R. J. Havel, H. A. Eder, and J. H. Bragdon. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34**:1345–1353 (1955).
21. K. M. Wasan, S. M. Cassidy, M. Ramaswamy, A. Kennedy, F. W. Strobel, S. P. Ng, and T. Y. Lee. A comparison of step-

gradient and sequential density ultracentrifugation and the use of lipoprotein deficient plasma controls in determining the plasma lipoprotein distribution of lipid-associated nystatin and cyclosporine. *Pharm. Res.* **16**:165–169 (1999).
22. M. Burstein, H. R. Scholnick, and R. Morfin. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **11**:583–595 (1970).
23. K. M. Wasan, R. E. Morton, M. G. Rosenblum, and G. Lopez-Berestein. Decreased toxicity of liposomal amphotericin B due to association of amphotericin B with high density lipoproteins: Role of lipid transfer protein. *J. Pharm. Sci.* **83**:1006–1010 (1994).
24. N. M. Pattnaik and D. B. Zilversmit. Interaction of cholesteryl ester exchange protein with human plasma lipoproteins and phospholipid vesicles. *J. Biol. Chem.* **254**:2782–2786 (1979).
25. L. J. Brunner, D. R. Luke, J. Lautersztain, *et al.* Single-dose cyclosporine pharmacokinetics in various biological fluids of patients receiving allogenic bone marrow transplantation. *Ther. Drug Monit.* **12**:134–138 (1990).
26. H. R. Betschart, W. R. Jondorf, and M. H. Bickel. Differences in adipose tissue distribution of basic lipophilic drugs between intraperitoneal and other routes of administration. *Xenobiotica* **18**:113–121 (1988).
27. C. B. Hesler, A. R. Tall, T. L. Swenson, P. K. Weech, Y. L. Marcel, and R. W. Milne. Monoclonal antibodies to the Mr 74,000 cholesteryl ester transfer protein neutralize all of the cholesteryl ester and triglyceride transfer activities in human plasma. *J. Biol. Chem.* **263**:5020–5023 (1988).
28. K. M. Wasan, G. A. Brazeau, A. Keyhani, A. C. Hayman, and G. Lopez-Berestein. Role of liposome composition and temperature on the distribution of amphotericin B in serum lipoproteins. *Antimicrob. Agents Chemother.* **37**:246–250 (1993).
29. J. Bolard, M. Seigneuret, and G. Boudet. Interaction between phospholipid bilayer membranes and the polyene antibiotic amphotericin B. Lipid State and cholesterol content dependence. *Biochim. Biophys. Acta.* **599**:280–293 (1980).
30. D. Sgoutas, W. MacMahon, A. Love, and I. Jerkunica. Interaction of cyclosporine A with human lipoproteins. *J. Pharm. Pharmacol.* **38**:583–588 (1986).
31. R. B. Shireman and J. F. Remsen. Imipramine associations with plasma components and its uptake by cultured human cells. *Life Sci.* **33**:2165–2171 (1983).