Differences in Lipoprotein Lipid Concentration and Composition Modify the Plasma Distribution of Cyclosporine

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Purpose. The purpose of this study was to define the relationship between lipoprotein (LP) lipid concentration and composition and the distribution of cyclosporine (CSA) in human plasma.

Methods. ^{3}H-CSA LP distribution was determined in normolipidemic human plasma that had been separated into different LP and lipoprotein-deficient plasma (LPDP) fractions by either affinity chromatography coupled with ultracentrifugation, density gradient ultracentrifugation or fast protein liquid chromatography. ^{3}H-CSA LP distribution (at a concentration of 1000 ng/ml) was also determined in patient plasma samples with defined dyslipidemias. Furthermore, ^{3}H-CSA LP distribution was determined in patient plasma samples of varying LP lipid concentrations. Following incubation, the plasma samples were separated into their LP and LPDP fractions by sequential phosphotungistic acid precipitation in the dyslipidemia studies and by density gradient ultracentrifugation in the specific lipid profile studies and assayed for CSA by radioactivity. Total plasma and lipoprotein cholesterol (TC), triglyceride (TG) and protein (TP) concentrations in each sample were determined by enzymatic assays.

Results. When the LP distribution of CSA was determined using three different LP separation techniques, the percent of CSA recovered in the LP-rich fraction was greater than 90% and the LP binding profiles were similar with most of the drug bound to plasma high-density (HDL) and low-density (LDL) lipoproteins. When ^{3}H-CSA was incubated in dyslipidemic human plasma or specific patient plasma of varying LP lipid concentrations the following relationships were observed. As the very low-density (VLDL) and LDL cholesterol and triglyceride concentrations increased, the percent of CSA recovered within the VLDL and LDL fractions increased. The percent of CSA recovered within the HDL fraction significantly decreased as HDL triglyceride concentrations increased. The percent of CSA recovered in the LPDP fraction remained constant except in hypercholesterolemic/hypertriglyceridemic plasma where the percent of CSA recovered decreased. Furthermore, increases in VLDL and HDL TG/TC ratio resulted in a greater percentage of CSA recovered in VLDL but less in HDL.

Conclusions. These findings suggest that changes in the total and plasma LP lipid concentration and composition influence the LP binding of CSA and may explain differences in the pharmacological activity and toxicity of CSA when administered to patients with different lipid profiles.

KEY WORDS: cyclosporine; lipoproteins; dyslipidemia.

INTRODUCTION

Transplantation success and the treatment of autoimmune diseases have benefited from the introduction of immunosuppressant drugs such as cyclosporine (CSA) (1–3). CSA is a cyclic polypeptide of fungal origin discovered in the early 1970's and approved for use in 1983 (4). It interacts with the intracellular protein cyclophillin, which inhibits the calcium dependent translocation of nuclear transcription factors, which are necessary for interleukin-2 gene transcription (3). Since, interleukin-2 is required for the proliferation of T-lymphocytes; CSA is capable at diminishing the immune response.

Despite the effectiveness of CSA as an immunosuppressant, the use of this drug is limited by renal toxicity, which is characterized by a rise in serum creatinine and a decrease in the glomerular filtration rate (5). Nephrotoxicity may occur in up to 75% of patients treated with CSA and is one of the major reasons CSA therapy is modified or discontinued. Other adverse effects of CSA administration include hypertension, hepatotoxicity, and gastrointestinal toxicity (5). The exact mechanism of CSA-induced toxicity is unclear but may involve an affect on the cytochrome P450 enzymatic systems in the liver and kidney causing nephropathy (6,7), the metabolism of the parent compound into nephrotoxic metabolites (8) and the interaction of CSA with cyclophillin (9).

Plasma lipoproteins are mainly involved in the transport of lipids but are also involved in other processes including immune reactions, coagulation and tissue repair (10,11). Traditionally lipoproteins are identified and separated based on their density into three broad groups: high-density lipoproteins (HDL), low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL), however, even within these groups there is heterogeneity in both particle size and lipid and protein composition. Lipoproteins also undergo complex metabolism, interacting with various tissues, enzymes circulating in the plasma and other lipoproteins. However, it is becoming apparent that lipoproteins also transport a number of hydrophobic compounds. For example, hydrophobic drugs such as amphotericin B (AmpB) bind to plasma lipoproteins (12) which appears to modify the pharmacokinetics, tissue distribution, and pharmacological activity of these compounds.

Cyclosporine (CSA) also has been shown to bind to lipoproteins upon incubation in human plasma (13–15) resulting in a modification of its pharmacological effect. Several investigators have reported decreases in CSA activity in patients that have hypertriglyceridemia (16,17) and increases in CSA toxicity in those with hypocholesterolemia (18). In addition, Lemaire and coworkers have suggested that the drug's availability to tissue and thus, its activity and toxicity may depend upon the specific class of lipoprotein to which the drug is bound (19). Taken together, these studies suggest that the effect of dyslipidemias (aberrant plasma lipoprotein and lipid concentrations) on the plasma distribution of CSA merit further investigation.

We conducted studies to determine whether the human plasma distribution of CSA was influenced by changes in lipoprotein lipid concentration. Since CSA is lipophilic our working

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hypothesis was that its association with different plasma lipoprotein classes would be influenced by the relative abundance of these lipid-protein macromolecules.

MATERIALS AND METHODS

Chemicals and Plasma

Radiolabeled CSA ([*mebmt*-β-^{3}H] Cyclosporin A; Specific Activity, 7.39 mCi/mg) was purchased from Amersham Life Science (Buckinghamshire England). Sodium bromide was purchased from Sigma Chemical Company (St. Louis, MO). Human plasma was obtained from the Vancouver Red Cross (Vancouver, British Columbia, Canada) and St. Paul's Healthy Heart Program (Vancouver, British Columbia, Canada). Ten μl of 0.4 M ethylenediaminetetraacetic acid pH 7.1 (EDTA, Sigma Chemical Company) was added to 1.0 ml of whole blood. For all CSA plasma distribution studies, tritium labeled CSA was reconstituted in a 100% ethanol. The volume of ethanol required to incubate 1000 ng/ml of CSA did not modify lipoprotein concentration or composition.

Lipoprotein Separation

Density Gradient Ultracentrifugation

The plasma was separated into its HDL, LDL, VLDL, and lipoprotein deficient plasma (LPDP) fractions by density-gradient ultracentrifugation (20,21). Briefly, human plasma (2.8 ml) samples were placed in centrifuge tubes and their density solvents were readjusted to 1.25 g/ml by the addition of sodium bromide. Once the sodium bromide was dissolved into the plasma, 2.8 ml of the highest density sodium bromide solution (density of 1.21 g/ml, which represents the HDL fraction) were layered onto the plasma solution. Then, 2.8 ml of the second sodium bromide solution (density of 1.063 g/ml which represents the LDL fraction) were layered onto the sample, followed by 2.8 ml of the third sodium bromide solution (density of 1.006 g/ml which represents the VLDL and chylomicron fraction). All sodium bromide solutions were kept at 4°C prior to the layering of the density gradient.

The sample-containing ultracentrifuge tubes were placed into individual titanium buckets (Beckman Canada), balanced, and capped. The buckets were then placed into their respective positions on a swinging bucket rotor (SW 41 Ti, Beckman Canada) and centrifuged at 40,000 rpm, at a temperature of 15°C for 18 hr (L8-80 M; Beckman Canada). After ultracentrifugation, the samples were carefully removed from the titanium buckets. Each density layer was removed using a pastuer pipette and the volume of each lipoprotein fraction measured.

Affinity Chromatography

Lipoproteins were separated into the HDL/LPDP and LDL/VLDL fractions by the LDL-Direct cholesterol chromatographic column at 4°C (22). This chromatographic column is a heparin-manganese polyacrylamide matrix, which separates lipoproteins based on their apolipoprotein content. Any plasma components that contain apolipoproteins B & E are retained onto the column while all other components are eluted. Once the virgin gel was fully hydrated with 1 ml of a preparatory solution (0.02% Sodium chloride + 0.002% chloramphenicol), plasma samples (200 μl) were placed onto the column followed by a HDL eluting agent (1 ml containing 0.02% sodium chloride + 0.002% chloramphenicol). The flow through fraction, which contains HDL (1.2 ml), was collected. The LDL/VLDL eluting agent (containing 2.9% sodium chloride + 0.002% chloramphenicol) was next placed onto the column and the LDL/VLDL fraction (2.4 ml) was collected. Subsequently, LDL was separated from VLDL and HDL from the LPDP fraction by density gradient ultracentrifugation as described previously (21,22).

Sequential Phosphotungstic Acid (PTA) Precipitation

Plasma was separated into its VLDL, LDL and HDL fractions by sequential precipitation. To precipitate VLDL, 12.5 ul of PTA reagent (4% (wt/v) tungstophosphoric-phosphoric acid) was added to 0.5 ml of plasma. The sample was mixed thoroughly, then incubated for 2 minutes at room temperature. Following this, 12.5 ul of 2 M $MgCl_2$ was added, the sample mixed thoroughly, then incubated at 4°C for 30 minutes. Following incubation, the sample was centrifuged at 14,000 g for 15 minutes. An aliquot of the supernatant was removed to determine the amount of radioactivity. To precipitate LDL and VLDL, the procedure was as described above except 50 ul of PTA reagent was added to the sample. The precipitate was collected by centrifugation at 4°C at 3000-× g for 30 minutes and resuspended in 0.5 ml of 0.5 M Na_2CO_3. An aliquot of both pellet and supernatant was removed to determine the amount of radioactivity present. To precipitate HDL, a 0.5-ml aliquot of the supernatant left after precipitating the VLDL and LDL was used. To this, 450 ul of PTA reagent was added, incubated for 2 minutes at room temperature after which 38 ul 2 M $MgCl_2$ was added and the sample incubated for 30 minutes at 4°C. The precipitate formed was collected by centrifugation and resuspended as described above. The amount of radioactivity associated with each fraction was determined.

Fast Protein Liquid Chromatography (FPLC)

Lipoprotein separation was performed with the use of a gel filtration column connected to a fast protein liquid chromatography (FPLC) system at 4°C. Briefly, serum lipoproteins were separated by gel filtration chromatography utilizing a Superose 6 column (1.6 cm × 50 cm) (Pharamcia, Dorval, Quebec). Two hundred microliters of plasma containing radiolabeled CSA was applied to the column and 0.5 ml fractions were collected in a buffer of 0.15 M NaCl, 1 mM EDTA and 0.03% (w/v) sodium azide at a flow rate of 0.25 ml/min at room temperature. Each run would take 75 minutes in length. The column was monitored by absorbency at 280 nm and the elution of ^{3}H-CSA was followed by determining the amount of radioactivity in each column fraction (Beckman LS-9000, Liquid Scintillation Counter, Beckman Instruments, Fullerton, CA). The column was standardized with respect to elution times for VLDL, LDL, HDL and human serum albumin (Figure 1). In addition, the column was characterized with respect to cholesterol, triglycerides and phosphorous by the methods previous described.

To assure that the distribution of CSA found in each of these fractions was a result of its lipoprotein association and not a result of the density of the formulation or non-specific

binding to the chromatographic columns, the density and elution fraction of ^{3}H-CSA reconstituted in 100% ethanol was determined by ultracentrifugation, affinity chromatography and FPLC. The majority of ^{3}H-CSA (>90%) was found in the density range of 1.000–1.006 g/mL and recovered from the chromatographic columns suggesting that CSA distribution is not a function of formulation density or influenced by non-specific binding.

Determination of Cyclosporine (CSA), Lipoprotein Lipid and Protein Levels

HDL, LDL, VLDL, and LPDP fractions were analyzed for ^{3}H-CSA against external standard calibration curves (corrected for quenching and luminescence) using radioactivity. Total and lipoprotein cholesterol, triglyceride and protein concentrations were determined by enzymatic assay kits from Sigma Diagnostics (St. Louis Mo.) as previously described (21).

Data and Statistical Analysis

Correlation coefficients between the amount of CSA recovered within the VLDL, HDL and LDL plasma fractions and the amount of cholesterol and triglyceride within these fractions and plasma lipoprotein composition were determined using Pearson's Test (Figure 3 and Table 5). Differences in the plasma distribution of CSA following incubation in human plasmas with defined dyslipidemias and of varying lipid 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 $\pm$ standard deviation.

Determination of the Distribution of Cyclosporine (CSA) in Plasma Lipoproteins Using Different Lipoprotein Separation Techniques

To demonstrate that CSA lipoprotein distribution is not a function of the lipoprotein separation technique used, ^{3}H-CSA was incubated in normolipidemic human plasma for 60 minutes at 37°C. Following incubation the plasma was immediately cooled to 4°C using an ice-water bath to prevent any redistribution of drug during lipoprotein separation (24) and separated into its lipoprotein and lipoprotein-deficient plasma fractions by either affinity chromatography coupled with density gradient ultracentrifugation, density gradient ultracentrifugation, or FPLC at 4°C as described in the materials and methods section. The percent of CSA recovered within each fraction was determined by radioactivity.

Cyclosporine (CSA) Distribution Studies in Human Plasmas with defined Dyslipidemias

To assess if broad changes in plasma lipid concentrations modify the plasma lipoprotein distribution of CSA, ^{3}H-CSA at 1000 ng/ml was incubated [peak concentrations observed following intravenous administration (23)] in normolipidemic (total cholesterol = 100–200 mg/dl; total triglyceride = 100–200 mg/dl), hypercholesterolemic (total cholesterol = 250–300 mg/dl), hypoalphalipoproteinemic (HDL cholesterol < 35 mg/dl), hypertriglyceridemic (total triglyceride = 350–500 mg/dl) and hypercholesterolemic & hypertriglyceridemic (total cholesterol = 250–300 mg/dl, total triglyceride = 350–500 mg/dl) human plasma for 60 minutes at 37°C. Following incubation the plasma was separated into its lipoprotein and lipoprotein-deficient plasma fractions by sequential PTA precipitation and the percent of CSA recovered within each fraction was determined by radioactivity. Since sequential PTA precipitation does not over estimate HDL cholesterol concentration at 4°C as it does at 21°C and 37°C (HDL cholesterol concentration at 4°C, 52.6 +/− 0.8; 21°C, 54.5 +/− 1.1; 37°C, 56.1 +/− 1.2 mg/dl; $p < 0.05$ vs. 4°C), following incubation for 60 minutes at 37°C the plasma was placed in an ice-water bath and cooled to 4°C prior to precipitation.

Cyclosporine (CSA) Distribution Studies in Specific Human Plasmas of Varying Lipoprotein Concentration and Composition

To gain an understanding on how lipoprotein lipids specifically influence the plasma distribution of CSA, ^{3}H-CSA was incubated in plasma from three different patients of varying total and lipoprotein cholesterol and triglyceride concentrations (Table 2) and composition (Table 3) for 60 minutes at 37°C. Following incubation, the plasma was cooled to 4°C and separated into its lipoprotein and lipoprotein-deficient fractions by density gradient ultracentrifugation and CSA distribution was determined by radioactivity.

RESULTS

Cyclosporine (CSA) Plasma Lipoprotein Distribution Using Different Lipoprotein Separation Techniques

To determine if CSA plasma lipoprotein distribution may be altered when using different lipoprotein separation techniques, ^{3}H-CSA at 1000 ng/ml was incubated in normolipidemic human plasma for 60 minutes at 37°C. Following incubation, the plasma was separated into its different lipoprotein and lipoprotein-deficient plasma fractions by either affinity chromatography coupled with ultracentrifugation, density gradient ultracentrifugation, or FPLC (Figure 1). As reported in Figure 2, only within the LDL fraction did the density gradient ultracentrifugation and FPLC method recover significantly less CSA than the affinity chromatography coupled with ultracentrifugation method. In the VLDL fraction only, the FPLC method significantly recovered more CSA than the affinity chromatography coupled with ultracentrifugation method. However, for all three methods, the over-all pattern of CSA recover was similar with most of the drug recovered in the HDL, LDL and VLDL fractions and less than 10% of the drug was recovered within the LPDP fraction (data not shown). Based on these findings, subsequent incubation studies used density gradient ultracentrifugation to separate lipoprotein and lipoprotein-deficient plasma fractions.

Distribution of Cyclosporine (CSA) Following Incubation in Human Plasmas with Defined Dyslipidemias

A significantly greater percentage of CSA was recovered in the VLDL/LDL fraction following the incubation of ^{3}H-CSA at 1000 ng/ml in hypercholesterolemic, hypertriglyceridemic,

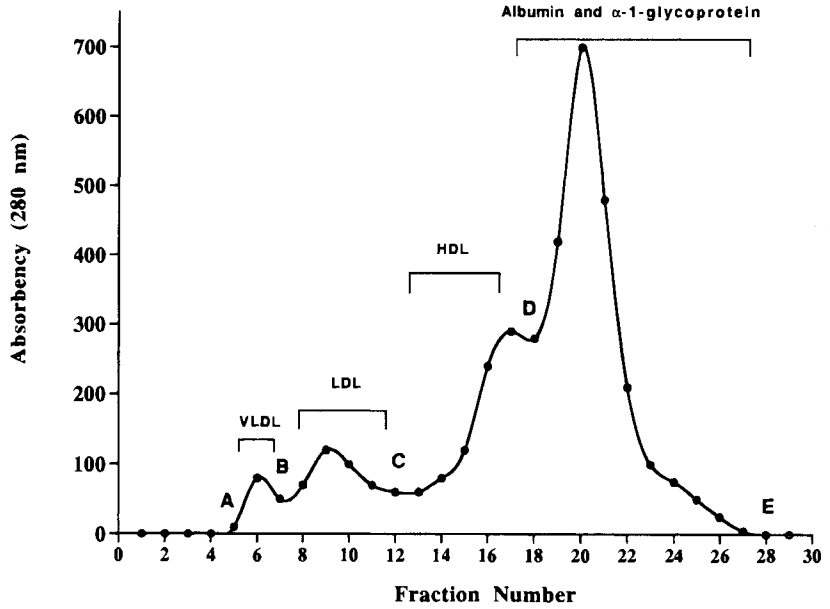

Fig. 1. Representative chromatogram of Fast Protein Liquid Chromatography separation of human plasma lipoproteins and albumin. Absorbency of lipoprotein phospholipids and aqueous proteins at 280 nm. Two hundred microliters of human plasma was injected onto a gel filtration column and 0.5 ml fractions were collected in a buffer of 0.15 M NaCl, 1 mM EDTA and 0.03% (w/v) sodium azide at a flow rate of 0.25 ml/minute at 4°C. Each run takes 75 minutes in length. The very low-density lipoprotein fraction was collected in fractions 5–7 (A to B), low-density lipoprotein fraction was collected in fractions 8–12 (B to C), high-density lipoprotein fraction was collected in fractions 13–17 (C to D), and the aqueous protein fraction which contains albumin and α-1-glycoprotein was collected in fractions 18–28 (D to E).

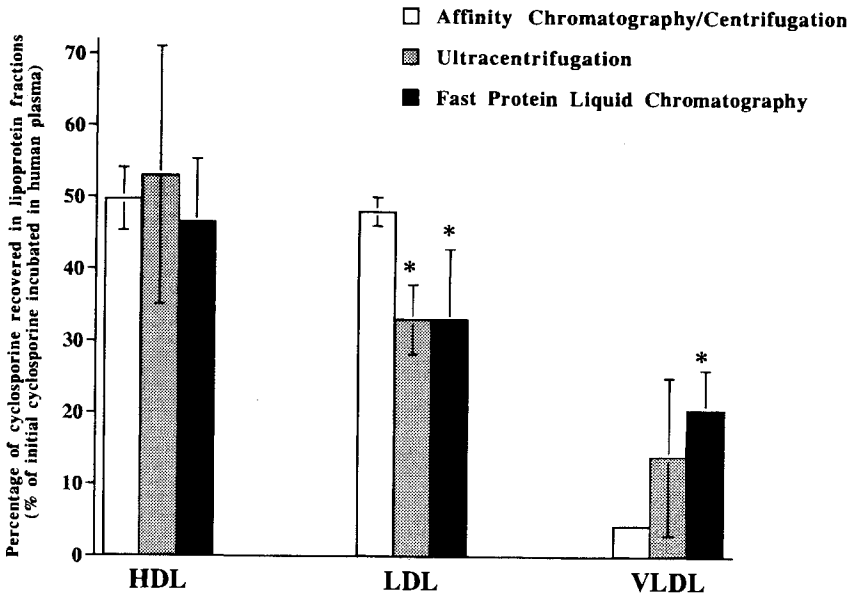

Fig. 2. Percent recovery of ^{3}H-cyclosporine (^{3}H-CSA) in high-density (HDL), low-density (LDL), and very low-density (VLDL) lipoprotein fractions. ^{3}H-CSA at 1000 ng/ml was incubated in normolipidemic human plasma for 60 minutes at 37°C. Following incubation the plasma was partitioned into its different lipoprotein subclasses by either affinity chromatography coupled with ultracentrifugation, density gradient ultracentrifugation, or fast protein liquid chromatography and the percent of CSA recovered in each of these fractions were determined by radioactivity. Data was expressed as mean +/− standard deviation (n = 6). * $p < 0.05$ vs. Affinity Chromatography/Centrifugation.

Table 1. Distribution of ^{3}H-Cyclosporine (^{3}H-CSA) at 1000 ng/ml Within Normolipidemic and Dyslipidemic Plasma from Different Patients Following Incubation for 60 Minutes at 37°C

Plasma Type	VLDL/LDL fraction %[a]	HDL fraction %	LPDP fraction %
Normolipidemic	31.9 ± 3.6	44.4 ± 4.2	19.7 ± 3.1
Hypercholesterolemic	46.3 ± 7.7*	20.9 ± 7.7***	20.9 ± 2.6
Hypoalphalipoproteinemic	42.7 ± 10.8	15.7 ± 3.9***	20.9 ± 3.6
Hypertriglyceridemic	54.3 ± 13.1**	20.0 ± 4.6***	18.9 ± 7.3
Hypercholesterolemic + Hypertriglyceridemic	55.3 ± 9.2**	20.1 ± 3.7***	12.9 ± 5.0*

Note: Data expressed as mean ± standard deviation (n− = 6); *p < 0.05, **p < 0.01, ***p < 0.001 vs. normolipidemic patient. Following incubation plasma samples were assayed by radioactivity for ^{3}H-CSA in each of the lipoprotein and lipoprotein-deficient plasma fractions.
[a] percent of initial ^{3}H-CSA concentration incubated; LPDP, lipoprotein-deficient plasma; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; Plasma type, Normolipidemic (total cholesterol = 100–200 mg/dl and triglyceride = 100–200 mg/dl); Hypercholesterolemic (total cholesterol = 250–300 mg/dl); Hyptertriglyceridemic (total triglyceride = 350–500 mg/dl); Hypoalphalipoproteinemic (HDL cholesterol < 35 mg/dl); Hypercholesterolemic + Hypertriglyceridemic (total cholesterol = 250–300 mg/dl and triglyceride = 350–500 mg/dl). Total CSA Recovery was greater than 88%.

and hypercholesterolemic + hypertriglyceridemic plasma for 60 minutes at 37°C compared to normolipidemic plasma (Table 1). A significantly lower percentage of CSA was recovered in the HDL fraction following incubation in hypercholesterolemic, hypoalphalipoproteinemic, hypertriglyceridemic, and hypercholesterolemic + hypertriglyceridemic plasma compared to normolipidemic plasma (Table 1). A significantly lower percentage of CSA was recovered in the LPDP fraction following incubation in hypercholesterolemic + hypertriglyceridemic plasma compared to other plasmas tested and the total drug recovery was greater than 88% (Table 1).

Distribution of Cyclosporine (CSA) Following Incubation in Plasma from Patients with Varying Lipid Concentrations

The total plasma and lipoprotein cholesterol (esterified & unesterified) and triglyceride concentrations were determined for three patients (Table 2). Patient II had a significantly greater total plasma and lipoprotein cholesterol and triglyceride concentration than patient I did with the exception of HDL-triglyceride levels (Table 2). Patient III had a significantly greater total plasma and lipoprotein cholesterol and triglyceride concentration than patient I did with the exception of HDL-cholesterol levels (Table 2). Patient III had a significantly greater total plasma and lipoprotein cholesterol and triglyceride concentration than patient II did (Table 2).

Furthermore, the plasma lipoprotein composition was calculated for three patients (Table 3). Patient II had a significantly lower LDL total cholesterol (TC): total protein (TP) (wt/wt) ratio than patient I (Table 3). Patient III had a significantly higher VLDL total triglyceride (TG): TP and TG: TC and HDL TG: TC than patient I (Table 3). However, patient III had a significantly lower HDL and LDL TC: TP and TG: TC than patient I did and a significantly higher HDL TG: TC than patient II did (Table 3).

A significantly greater percentage of CSA was recovered within the LDL fraction following the incubation of ^{3}H-CSA at 1000 ng/ml in patient III plasma for 60 minutes at 37°C than following the incubation of ^{3}H-CSA in patient I's plasma (Table 4). However, a significantly lower percentage of CSA was recovered within the HDL fraction following the incubation of ^{3}H-CSA in patient III plasma than following the incubation in patient's I and II plasmas (Table 4).

When correlation's between the amount of CSA recovered within the VLDL, HDL and LDL plasma fractions and the amount of cholesterol and triglyceride within these fractions were calculated for all three patient plasmas the following relationships were observed. As VLDL cholesterol and triglyceride increase, the amount of CSA recovered within this fraction

Table 2. Total and Lipoprotein Plasma Cholesterol (Esterified + unesterified) and Triglyceride Concentrations from Three Different Patients

Patient profile	Very low density lipoproteins mg/dl	Low density lipoproteins mg/dl	High density lipoproteins mg/dl	Total mg/dl
Cholesterol (esterified + unesterified)				
Patient I	18.6 ± 1.4	62.9 ± 0.01	31.4 ± 0.02	112.9 ± 1.4
Patient II	51.7 ± 2.2*	85.4 ± 1.32*	42.1 ± 5.0*	179.2 ± 6.3*
Patient III	73.7 ± 6.4* **	128.3 ± 3.3* **	32.4 ± 4.1	234.5 ± 2.4* **
Triglyceride				
Patient I	36.2 ± 1.6	20.9 ± 0.1	22.2 ± 0.1	79.3 ± 1.6
Patient II	113.8 ± 5.6*	36.1 ± 4.4*	16.8 ± 5.8	166.7 ± 4.1*
Patient III	285.5 ± 10.0* **	69.4 ± 3.1* **	39.5 ± 11.1* **	394.4 ± 12.7* **

Note: Data is expressed as mean ± standard deviation; (n = 6).
* p < 0.05 vs. patient I profile.
**p < 0.05 vs. patient II profile.

Table 3. Plasma Lipoprotein Composition from Three Different Patients

Lipoprotein fraction	Patient I	Patient II	Patient III
Very Low Density Lipoproteins			
TC/TP (wt/wt)	2.4 ± 0.6	2.2 ± 0.5	1.8 ± 0.2
TG/TP (wt/wt)	4.0 ± 1.9	4.7 ± 1.2	6.1 ± 0.4*
TG/TC (wt/wt)	1.6 ± 0.4	2.2 ± 0.2	3.5 ± 0.5*
Low Density Lipoproteins			
TC/TP (wt/wt)	2.0 ± 0.4	1.0 ± 0.4*	0.9 ± 0.4*
TG/TP (wt/wt)	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.2
TG/TC (wt/wt)	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.1*
High Density Lipoproteins			
TC/TP (wt/wt)	0.21 ± 0.04	0.15 ± 0.04	0.09 ± 0.03*
TG/TP (wt/wt)	0.16 ± 0.02	0.11 ± 0.06	0.16 ± 0.06
TG/TC (wt/wt)	0.8 ± 0.1	0.8 ± 0.5	2.1 ± 0.5* **

Note: Data is expressed as mean ± standard deviation, (n = 6); *p < 0.05 vs. patient I profile; **p < 0.05 vs. patient II profile; TC, total cholesterol (esterified + unesterified); TG, total triglycerides; TP, total protein; wt/wt, weight/weight.

proportionally increases (Figure 3 I A&B r = 0.78). As LDL cholesterol and triglyceride increase, the amount of CSA recovered within this fraction proportionally increases (Figure 3 II A&B; r > 0.90). By contrast, as HDL triglyceride increases, the amount of CSA recovered within this fraction proportionally decreases (Figure 3 III B; r = 0.86).

When correlation's between CSA amount recovered in each lipoprotein fraction and lipoprotein composition were determined the following relationships were observed. As VLDL TG: TC increased, the amount of CSA recovered in VLDL proportionally increased (Table 5). When HDL TC: TP increased the amount of CSA recovered in HDL increased while when HDL TG: TC increased the amount of CSA recovered decreased (Table 5).

DISCUSSION

The purpose of these studies were to determine if alterations in plasma lipoprotein lipid concentrations, often exhibited by immunocompromised (25), heart transplant (26) and kidney transplant patients (27), modifies the plasma lipoprotein distribution of CSA and by which factor(s). We observed that increases in LDL lipid concentrations increased the amount of CSA recovered in this fraction and subsequently decreased the amount of CSA recovered within the HDL fraction without significantly altering the amount of drug recovered within the VLDL and LPDP fractions (Table 4).

Previous studies with the antifungal agent, AmpB, have suggested that an alteration in plasma lipid concentrations modify this drug's pharmacological behavior. Chavanet and coworkers have demonstrated that an increase in plasma triglyceride concentration lead to a reduction in AmpB toxicity (28). These findings suggested that the triglycerides, or their main vehicle in serum, chylomicrons, LDL and VLDL, were involved in the protective effect against AmpB toxicity. Souza and coworkers have further shown that a triglyceride-rich emulsion that behaves in vivo as chylomicrons was able to reduce the in vivo and in vitro toxicity of AmpB (29). In addition, we recently have shown enhanced AmpB-induced renal toxicity in intensive care patients who exhibited elevated serum LDL cholesterol levels (25).

Furthermore, a number of studies have supported the importance of plasma lipoprotein binding and concentration in influencing the therapeutic index of CSA. Lemaire and coworkers have observed enhanced antiproliferative effects of CSA when the drug was bound to LDL, which was not evident, when the drug was bound to either VLDL or HDL (19,30). Gardier and coworkers observed that heart transplant patients with high total plasma cholesterol levels demonstrated increased CSA association with plasma LDL and an increased CSA-induced renal toxicity compared to normolipidemic controls (26). In addition, Arnadottir and coworkers observed elevations in CSA-induced renal toxicity in kidney transplant patients who exhibited increases in plasma cholesterol concentration (27). These studies provide compelling evidence suggesting that plasma lipoprotein lipid levels have a major impact on influencing the efficacy and toxicity of CSA.

In the present study, we have observed that broad plasma dyslipidemias and specific increases in LDL and VLDL lipid levels resulted in an increasing amount of CSA recovered in these fractions and a significant decrease in the amount of CSA recovered in the HDL fraction. Furthermore, the amount of drug recovered in the non-lipoprotein fraction remained unchanged (Tables 1 and 4). These findings suggest that CSA lipoprotein distribution may be partially regulated by plasma lipoprotein cholesterol and to a lesser extent triglyceride concentrations.

Table 4. Distribution of ^{3}H-Cyclosporine (^{3}H-CSA) at 1000 ng/ml Within Plasma from Three Different Patients following Incubation for 60 Minutes at 37°C. Following Incubation Plasma Samples were Assayed by Radioactivity for Drug in Each of the Lipoprotein and Lipoprotein-Deficient Plasma Fractions

Patient profile	VLDL fraction %[a]	HDL fraction %	LDL fraction %	LPDP fraction %	Percent recovery %[b]
Patient I	12.6 ± 6.0	47.2 ± 5.4	26.8 ± 4.4	7.6 ± 2.7	94.1 ± 6.5
Patient II	16.6 ± 6.1	40.6 ± 7.8	33.9 ± 4.9	5.3 ± 1.1	96.5 ± 4.7
Patient III	19.4 ± 6.6	28.2 ± 2.3*,**	41.1 ± 2.5*	6.9 ± 1.4	95.5 ± 5.9

Note: Data expressed as mean ± standard; *p < 0.05 vs. Patient I, **p < 0.05 vs. Patient II (n = 6).

[a] percent of initial ^{3}H-CSA concentration.

[b] percent of initial drug incubated; LPDP, lipoprotein-deficient plasma; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

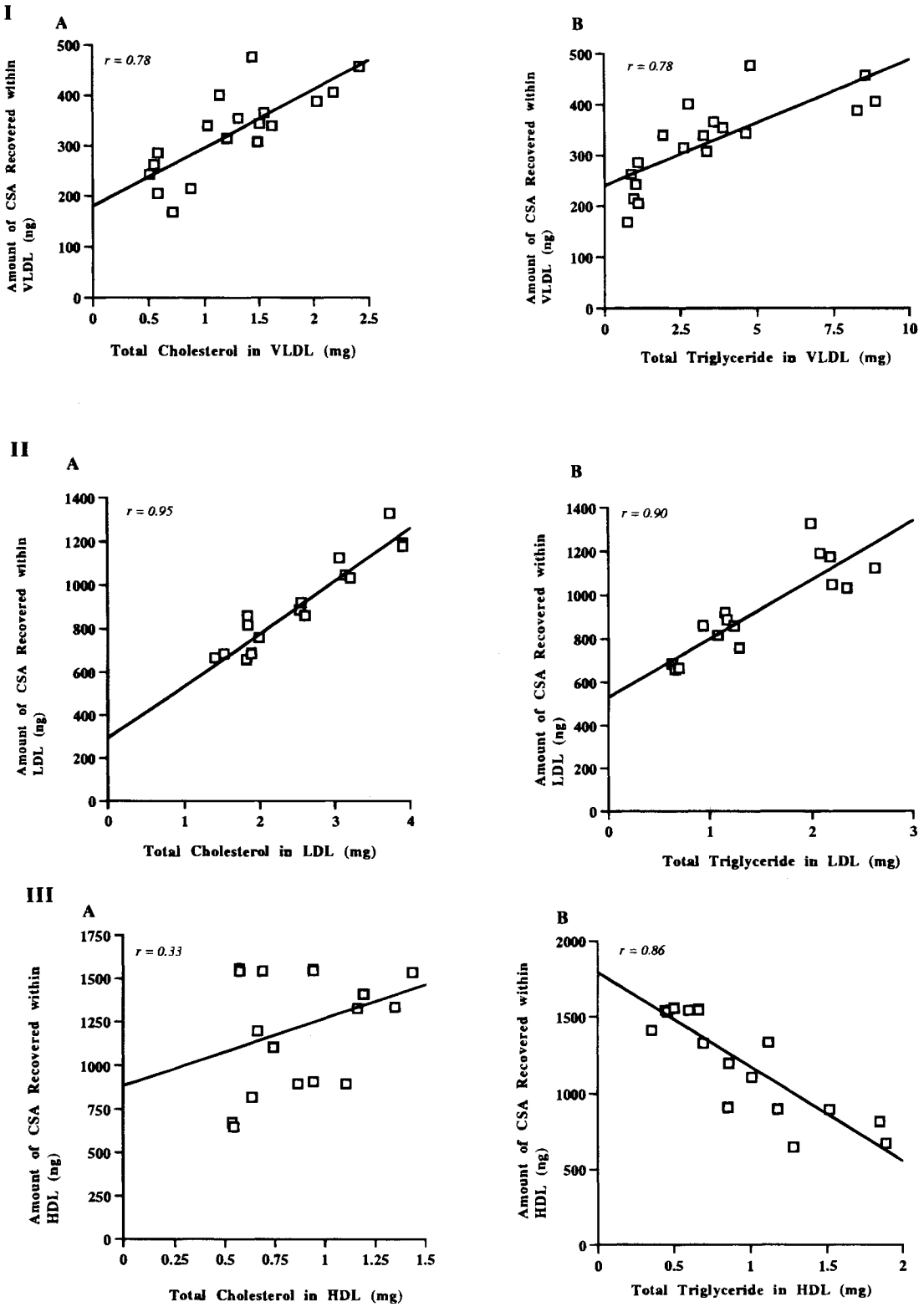

Fig. 3. The amount of ^{3}H-cyclosporine (^{3}H-CSA) recovered within the **I.** Very low-density lipoprotein (VLDL) fraction versus [A] the amount of cholesterol in VLDL and [B] the amount of triglyceride in VLDL, **II.** Low-density lipoprotein (LDL) fraction versus [A] the amount of cholesterol in LDL and [B] the amount of triglyceride in LDL, or **III.** High-density lipoprotein (HDL) fraction versus [A] the amount of cholesterol in HDL and [B] the amount of triglyceride in HDL following the incubation of ^{3}H-CSA at 1000 ng/ml for 60 minutes at 37°C in different human plasmas of varying total and lipoprotein lipid concentrations. Data represents individual incubations within three different patients (n = 6 for each patient; total n = 18).

Table 5. Correlation Coefficients Between the Amount of ^{3}H-Cyclosporine (^{3}H-CSA) Recovered in Each Lipoprotein Fraction with Plasma Lipoprotein Composition

	CSA-VLDL	CSA-LDL	CSA-HDL
TC/TP	−0.43 ns	−0.55 ns	0.87[a]
TG/TP	0.57 ns	−0.34 ns	−0.30 ns
TG/TC	0.82[a]	0.56 ns	−0.81[a]

Note: Pearson correlation coefficients between ^{3}H-Cyclosporine (^{3}H-CSA) associated with lipoproteins and plasma lipoprotein composition. Results are expressed as r (Pearson correlation coefficient) values with significance.

[a] $p < 0.05$ and ns, not significant. VLDL, very low-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; TC, total cholesterol; TG, total triglyceride; TP, total protein.

It further suggests the redistribution of drug from one lipoprotein class (HDL) to another (LDL or VLDL) could be influenced by different disease states (25–27,31) and adjunct therapies such as Intralipid infusion (32), where lipoprotein plasma concentrations and composition are altered. In addition, it appears that variations in total plasma and lipoprotein lipid concentration does not influence the recovery of the drug in the LPDP fraction. This confirms that CSA binding to aqueous plasma proteins (i.e. albumin and α-1-glycoprotein) is not a major fact in regulating the overall plasma distribution of CSA (13).

We have further observed that although HDL cholesterol did not increase and HDL triglyceride concentration increased 1.8-fold from patient I through to patient III (Table 2) the percent of CSA recovered within this fraction significantly decreased 1.5-fold (Table 4). In addition, although VLDL cholesterol and triglyceride concentrations increased 4-fold and 8-fold respectively (Table 2), the percent of CSA recovered within this fraction increased, but not significantly (Table 4).

However, in these same plasmas, when plasma LDL cholesterol and triglyceride concentrations increased 2-fold and 3-fold respectively (Table 2), the percent of CSA recovered within this fraction increased 1.5-fold (Table 4). This resulted in a significant and proportional increase in the amount (Figure 3 II A&B) CSA recovered within the LDL fraction.

In addition, unlike the work of Gardier and coworkers, we observed that increasing the TG: TC ratio within VLDL and HDL (Table 5) resulted in more CSA recovered in the VLDL fraction but less CSA recovered in the HDL fraction (Table 4). We further observed that increases in the HDL TC: TP ratio increased the amount of drug recovered in the HDL fraction (Table 5). These findings suggest that not only lipid mass (cholesterol and triglyceride) and lipoprotein composition but also the type of lipoprotein in which these changes occur is another possible factor in determining to which lipoprotein CSA binds. However, since previous studies have suggested that erythrocytes and leukocytes largely carry CSA when incubated in whole blood (33), future work will address how changes in the lipid profile would affect the whole blood distribution of CSA.

In conclusion we have determined that CSA predominantly associates with lipoproteins upon entrance into the plasma component of the bloodstream. Furthermore, not only the relative levels of individual lipoproteins but also their lipid composition define the distribution of CSA among plasma lipoproteins. Since transplantation patients exhibit lipid disturbances, including hypocholesterolemia and hypertriglyceridemia (26,27), these results may provide an explanation for the unpredictable and inconsistent pharmacokinetics and pharmacodynamics of CSA following administration.

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