Modifications in Lipoprotein Surface Charge Alter Cyclosporine A Association with Low-Density Lipoproteins

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Purpose. The purpose of this study was to examine the influence of lipoprotein surface charge on the plasma distribution of cyclosporine A (CSA).

Methods. Phosphatidylinositol (PI; 40 μmol) was administered intravenously to rabbits. Blood was removed 10 min after injection and plasma was retrieved. Radiolabeled CSA ([^{3}H] CSA) at a concentration of 1000 ng/mL was incubated for 60 min at 37°C in control and PI-treated rabbit plasma. After incubation, plasma was separated into its lipoprotein and lipoprotein-deficient plasma (LPDP) fractions by density gradient ultracentrifugation, and the percentage of [^{3}H]CSA recovered in each fraction was determined by radioactivity. To determine lipoprotein surface charge within control and PI-treated plasma, the zeta potential of each lipoprotein fraction was measured. The effect of PI on lipoprotein surface charge was further confirmed by gel electrophoresis.

Results. PI treatment caused low-density lipoprotein (LDL) fraction to migrate further on the agarose gel, indicative of an increased negative surface charge. Zeta potential analysis further showed that LDL particles had a surface potential of -11.4 ± 1.9 mV and -17.4 ± 3 mV in control and PI-treated groups, respectively. A greater percentage of [^{3}H]CSA was recovered within the LDL ($16.4 \pm 1.1\%$ vs. $7.7 \pm 2.1\%$; $n = 3$; $p < 0.05$) fraction after incubation in PI treated than in control plasma, respectively.

Conclusion. These findings suggest that modifications in lipoprotein surface charge alter CSA distribution within the LDL plasma fraction.

KEY WORDS: cyclosporine A; plasma lipoproteins; surface charge; phosphatidylinositol.

INTRODUCTION

Plasma lipoproteins are macromolecular complexes of lipid and protein that are mainly involved in the transport of lipids through the vascular and extravascular body fluids (1) and other processes, including immune reactions, coagulation, and tissue repair (2–4). Recently, plasma lipoproteins have been implicated in the transport of a number of water-insoluble agents, resulting in the modification of their pharmacokinetics, tissue distribution, and pharmacologic activity (5,6).

Cyclosporine A (CSA) is an effective immunosuppressant used in the treatment of a number of autoimmune diseases as well as in human transplantation (7,8). Despite its effectiveness, CSA therapy is limited by renal toxicity, characterized by a rise in serum creatinine and a decrease in the glomerular filtration rate (9). Furthermore, it has been well documented that CSA associates with human lipoproteins (10–13). Changes in the lipid concentration and composition of plasma lipoproteins, including diseases such as hypothyroidism and liver disease (14), alter the profile of CSA–lipoprotein association (13). These findings suggest that the association of hydrophobic drugs, such as CSA with lipoproteins, can significantly influence the pharmacologic and pharmacokinetic properties of the drug as well as its relative toxicity (5,6). Decreased CSA efficacy has been associated with hyperlipidemia, particularly hypertriglyceridemia (15) whereas increased toxic effects of CSA have been associated with hypocholesterolemia (16). In addition, the interaction of CSA with high-density (HDL) and low-density lipoproteins (LDL) significantly reduces the renal clearance and extent of renal tissue distribution of the drug in an isolated perfused rat kidney model (17).

Our group has previously reported that not only the relative levels of individual lipoproteins but also their lipid composition define the distribution of CSA among plasma lipoproteins (13). However, to date, few studies have examined other lipoprotein physical chemical characteristics that influence the lipoprotein association of hydrophobic drugs like CSA. One such characteristic is lipoprotein surface charge. Previous studies have demonstrated that HDL and LDL have high and low surface polarity, respectively (18) and that an intravenous injection of phosphatidylinositol (PI) into fasted rabbits causes a significant increase in the net negative surface charge of these lipoproteins (19). Thus, we hypothesized that differences in lipoprotein surface charge might be a possible rationale for differences in lipoprotein association of CSA. The current study examined the influence of lipoprotein surface charge on the plasma distribution of CSA.

MATERIALS AND METHODS

Chemicals and Plasma

PI and radiolabeled CSA ([*mebmt*-β-^{3}H] Cyclosporin A; specific activity, 6.64 mCi/mg) were purchased from Amersham Life Science (Buckinghamshire, UK). Drug-free vehicles used to reconstitute ^{3}H-CSA did not alter lipoprotein lipid and protein composition and concentration (13,20). Methanol, tetrahydrofuran, and other organic solvents were purchased from Fisher Scientific Canada (Toronto, Ontario, Canada). Sodium bromide was purchased from Sigma Chemical Company (St. Louis, MO, USA). Normolipidemic-fasted human plasma was obtained from the Vancouver Red Cross (Vancouver, British Columbia, Canada). Ten microliters of 0.4 M ethylenediaminetetraacetic acid pH 7.1 (Sigma Chemical Company) was added to 1.0 mL of whole blood.

Lipoprotein Separation

Rabbit plasma was separated into its lipoprotein and lipoprotein deficient fractions by step-gradient ultracentrifugation (13,20). Briefly, plasma samples (3.0 mL) were placed into centrifuge tubes, and their solvent densities were adjusted to 1.25 g/mL by the addition of solid sodium bromide (0.34 g/mL of plasma). Once the sodium bromide had dis-

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solved into the plasma, 2.8 mL of the highest density sodium bromide solution (density of 1.21 g/mL, which represents the HDL fraction) was layered on top of the plasma solution. Then, 2.8 mL of the second sodium bromide solution (density of 1.063-g/mL, which represents the LDL fraction) was layered on top of the sample, followed by 2.8 mL of the third sodium bromide solution (density of 1.006 g/mL, which represents the very low-density lipoprotein [VLDL] and chylomicron fraction). Upon completion of layering with the sodium bromide density solutions, four distinct regions of progressively greater densities (from top to bottom of the tube) were observed (20). All sodium bromide solutions were kept at 4°C before the layering of the density gradient. The centrifuge tubes were placed in an SW 41 Ti swinging bucket rotor (Beckman Canada) and centrifuged at 40,000 rpm (288,000 g; k factor = 128), at a temperature of 15°C for 18 h (L8-80 M; Beckman Canada). After ultra centrifuge (UC), each density layer was removed using a Pasteur pipette and the volume of each lipoprotein fraction measured.

^{3}H-CSA, Lipid, and Protein Quantification

^{3}H-CSA recovery within each lipoprotein and lipoprotein-deficient fraction was determined by scintillation counting and calculating the amount of ^{3}H-CSA within each fraction using external calibration/quenching standard curves (13). Lipoprotein plasma triglyceride, cholesterol, and protein concentrations were determined by enzymatic assays purchased from Sigma Chemical Co. as previously described (13,20).

Zeta Potential Measurement and Lipoprotein Size Determination

To determine lipoprotein surface charge within control and PI-treated plasma, the zeta potential of each lipoprotein fraction was determined as previously described (19). The effect of PI on lipoprotein surface charge was further confirmed by gel electrophoresis as previously described (18).

Experimental Design

PI (40 μmol) was administered intravenously via the marginal ear vein to female New Zealand White Rabbits (3–3.5 kg). Blood was removed 10 min after injection and plasma was retrieved. Radiolabeled CSA ([^{3}H] CSA) at a concentration of 1000 ng/mL was incubated for 60 min at 37°C in control and PI-treated rabbit plasma. After incubation plasma was separated into its lipoprotein and lipoprotein-deficient plasma (LPDP) fractions by density gradient ultracentrifugation and the percentage of [^{3}H]CSA recovered in each fraction was determined by radioactivity.

After incubation, the plasma samples were partitioned into different densities using step-gradient or sequential density UC and each density fraction was assayed for drug by high-performance liquid chromatography or radioactivity. In human plasma the 1.0–1.006 g/mL density fraction represents VLDL and chylomicrons, the 1.006–1.063 g/mL density fractions represents LDL, the 1.063–1.21 g/mL density fraction represents HDL, and the greater than 1.21 g/mL density fraction represents the LPDP fraction composed of mostly albu-

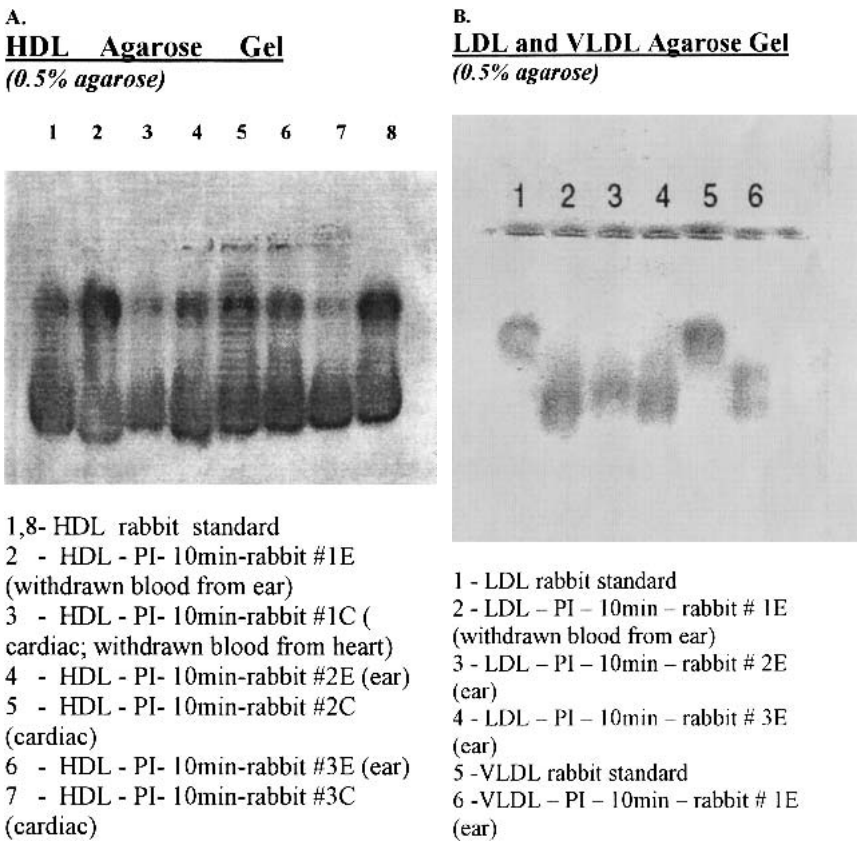

Fig. 1. Representative gel electrophoresis of control and PI-treated HDL (A), LDL, and VLDL (B) particles taken from the ear vein and/or cardiac puncture.

Table I. Lipoprotein Particle Surface Charge as Measured by Zeta Potential in Control and PI-Treated Rabbit Plasma

Lipoprotein particle	Control plasma (mV)	PI-treated plasma (mV)
HDL	−18.9 ± 1.4	−18.1 ± 2.8
LDL	−11.4 ± 1.9	−17.4 ± 3.0*
VLDL	−10.5 ± 1.5	−18.3 ± 1.9*

Note: Data reported as mean ± standard deviation; n = 3.
* $p < 0.05$ vs. control plasma.

min and alpha-1 glycoprotein. Furthermore, the lipoprotein subfractions from control and PI-treated plasma were further analyzed for their lipid and protein content, particle surface charge and particle size.

Statistical Analysis

Differences in drug distribution between control and PI-treated plasma were determined by a one-way analysis of variance (InStat; GraphPad Software). Critical differences were assessed by Tukey posthoc tests. Differences were considered significant if p was < 0.05. All data are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

A number of preliminary studies have been completed to determine which factors influence CSA association with specific lipoproteins. Our laboratory has previously reported that not only the relative levels of individual lipoproteins but also their lipid composition define the distribution of CSA among plasma lipoproteins (13). Furthermore, we have observed that the distribution of CSA among lipoproteins is partially influenced by a plasma glycoprotein responsible for the transfer lipoprotein core lipids (e.g., cholesteryl esters, triglycerides) between lipoproteins fractions, cholesteryl ester transfer protein (21). However, to date few studies have investigated the importance of lipoprotein surface charge on the association of CSA with specific lipoprotein particles.

In the present study, PI treatment caused LDL and VLDL fractions to migrate further on the agarose gel, indicative of an increased negative surface charge (Fig. 1). Zeta potential analysis further showed that LDL particles had a surface potential of −11.4 ± 1.9 mV and −17.4 ± 3 mV in control and PI-treated groups respectively (Table I). Minimal changes in particle size (Table II), lipid and protein content, hydrophobic volume, and physical structure using transmission electron microscopy (data not shown) with control and

Table II. Lipoprotein Particle Size within Control and PI-Treated Rabbit Plasma

Lipoprotein particle	Control plasma (nm)[a]	PI-treated plasma (nm)
HDL	10.6 ± 1.0	10.5 ± 0.2
LDL	22.7 ± 0.8	23.8 ± 3.3
VLDL	35.5 ± 2.2	40.3 ± 1.5*

Note: Data reported as mean ± standard deviation; n = 3.
* $p < 0.05$ vs. control plasma.
[a] Particle mean diameter.

Table III. Plasma Lipoprotein Distribution of [^{3}H]CSA (1000 ng/ml) after 60 Min of Incubation at 37 °C in Control and PI-Treated Rabbit Plasma

Lipoprotein particle	Control plasma (%)[a]	PI-treated plasma (%)
HDL	75.1 ± 5.3	71.1 ± 2.0
LDL	7.7 ± 0.4	16.4 ± 0.6*
VLDL	2.8 ± 0.8	1.2 ± 0.2*
LPDP	10.1 ± 0.8	8.0 ± 0.1*

Note: Data reported as mean ± standard deviation; n = 3.
* $p < 0.05$ vs. control plasma.
[a] Percent of initial [^{3}H]CSA concentration incubated in plasma.

PI-treated lipoproteins were observed. A greater percentage of [^{3}H]CSA was recovered within the LDL fraction after incubation in PI-treated than in control plasma (Table III). Taken together, these findings suggest that increases in LDL surface charge resulted in a greater concentration of CSA recovered within this lipoprotein fraction.

Understanding which physicochemical properties of LDL influence CSA association with this lipoprotein has pharmacologic implications. Hyperlipidemia, which is common in transplant recipients, is thought to augment the toxic and/or pharmacologic effects of CSA by modifying its association with plasma proteins and lipoproteins (13,14). Decreased CSA efficacy has been associated with hyperlipidemia, particularly hypertriglyceridemia (15) whereas increased toxic effects of CSA have been associated with hypocholesterolemia (16). In addition, the interaction of CSA with HDLs and LDLs significantly reduced the renal clearance and extent of renal tissue distribution of the drug in an isolated perfused rat kidney model (17). Recently, our laboratory has shown that the uptake and toxicity of CSA within LLC-PK_1 pig kidney cells was effectively reduced with elevated LDL concentrations but showed a significant increase when incubated with elevated concentrations of apoA-I (22). Furthermore, increasing VLDL and HDL concentrations slightly reduced CSA toxicity and uptake but showed little effect with increased incubation time. Triglyceride and cholesterol, the respective major components of VLDL and LDL, did not alter CSA uptake or toxicity under the conditions tested. Take together these data suggest a possible relationship between CSA-induced toxicity and the nature of the associated lipoprotein (22).

In conclusion, these findings suggest that modifications in lipoprotein surface charge alter CSA distribution within the LDL plasma fraction and may provide a further explanation to why CSA preferentially associates with this lipoprotein upon incubation in plasma.

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