Effects of Low and High Density Lipoproteins on Renal Cyclosporine A and Cyclosporine G Disposition in the Isolated Perfused Rat Kidney

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Received April 14, 1997; accepted July 7, 1997

Purpose. This study investigated the effects of low (LDL) and high density lipoproteins (HDL) on renal cyclosporine A (CsA) and cyclosporine G (CsG) disposition in the isolated perfused rat kidney model.

Methods. Kidneys were perfused with CsA or CsG in perfusion medium containing 6% protein, bovine serum albumin only (BSA) (Control), LDL (200 mg/dl) and BSA, or HDL (200 mg/dl) and BSA. *In vitro* protein binding studies were conducted with CsA and CsG in the same media.

Results. The unbound fractions (f_u) of CsA and CsG were significantly reduced with LDL and HDL in the perfusion media. In the presence of LDL, f_u for CsA and CsG was 3.9% and 5.9%, respectively. With HDL, f_u was 2.1% for CsA and 1.8% for CsG. f_u for the controls was 14.7% for CsA and 11.9% for CsG. Renal clearance (CL_R) of CsA and CsG was significantly reduced when perfused with perfusion medium containing LDL and HDL. LDL and HDL had similar effects on reducing CsA and CsG CL_R, and were ~four-fold lower when compared to controls (~0.006 Vs. 0.023 ml/min). Renal CsA and CsG tissue (whole organ, cortex and medulla) concentrations were lower than corresponding controls when perfused with LDL or HDL.

Conclusions. The interaction of CsA and CsG with LDL and HDL significantly reduced the CL_R and extent of renal tissue distribution of both compounds.

KEY WORDS: renal clearance; cyclosporine A; cyclosporine G; low density lipoprotein; high density lipoprotein; isolated perfused kidney.

INTRODUCTION

Cyclosporine A (CsA) is a highly lipophilic, cyclic undecapeptide that is used to prevent graft rejection in solid organ transplantation and graft-*versus*-host disease in bone marrow transplantation, and to prevent or retard the progression of autoimmune diseases (1). Nephrotoxicity is a major adverse effect of CsA that limits its use (2). Cyclosporine G (CsG) is a naturally occurring structural analog of CsA that has been shown to be similar to CsA in immunosuppressive activity and potency, but less nephrotoxic (3). The differences in nephrotoxicity between CsA and CsG could be due to differences in drug disposition in the kidney.

In whole blood, CsA has been shown to interact with erythrocytes (58%), lipoproteins (27%), granulocytes (5%), lymphocytes (4%), and other proteins (5%) (4). In plasma, CsA interacts principally with lipoproteins (4), ~45–60% with high density lipoprotein (HDL), 30–35% with low density lipoprotein (LDL), and smaller amounts with intermediate, very low density lipoprotein (VLDL), and chylomicrons (5,6). Only about 10% of bound CsA is associated with nonlipoprotein proteins. In human plasma, the unbound fraction (f_u) of CsA ranges from 2 to 12% (5,7,8).

Lipoproteins have been observed to play a major role in the *in vivo* transport of many lipophilic compounds and have been implicated in altering the disposition and nephrotoxicity of CsA in transplant patients (9). In addition, patients receiving CsA treatment have often developed hyperlipidemia and/or alterations in lipoprotein profiles (10,11). In view of these observations, the purpose of this study was to investigate the effects of low and high density lipoproteins on renal CsA and CsG disposition in the isolated perfused rat kidney.

MATERIALS AND METHODS

Drugs and Chemicals

Cyclosporine A, cyclosporine G, and cyclosporine D (CsD) powder were obtained from Sandoz Pharmaceuticals Corporation, East Hanover, NJ. Stock CsA and CsG solutions were prepared by dissolving CsA or CsG powder in 10% ethanol in polyethylene glycol 200 at a concentration of 1 mg/ml for use in the preparation of drug perfusion media. Bovine serum albumin-Fraction V (BSA) was purchased from ICN Biomedicals, Inc., Aurora, OH. Nutrients used in the preparation of the perfusion medium were D-glucose (Sigma Chemical Co., St. Louis, MO), and a mixture of twenty essential L-amino acids (8.5% Travesol® Injection, Baxter Healthcare Corp., Deerfield, IL; Sigma Chemical Co., St. Louis, MO). ^{14}C-Inulin with known specific activity and radiochemical purity >98% was obtained from ICN Radiochemicals, Irvine, CA. The low (LDL) and high density lipoproteins (HDL) used in this investigation were isolated from the sera of fresh bovine whole blood (Greater Omaha Packing Co., Omaha, NE).

Animals

Male, Sprague-Dawley rats (Sasco, Inc., Omaha, NE), 300 to 400 g, were used. The rats were housed under controlled conditions at 21 ± 1°C on a 24 hour, alternating light– (0600–1800) dark cycle with free access to standard laboratory chow and tap water.

Isolated Perfused Rat Kidney System

The isolated perfused rat kidney preparation used was a recirculating system adapted as described by Nishiitsutsuji-Uwo *et al.* (12). The perfusion medium was continuously oxygenated

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ABBREVIATIONS: CsA, cyclosporine A; CsD, cyclosporine D; CsG, cyclosporine G; BSA, bovine serum albumin; C_f, unbound drug concentration in perfusate; CL_R, renal clearance; $FE_{glucose}$, fraction of glucose excreted; FE_{sodium}, fraction of sodium excreted; f_u, unbound drug fraction; GFR, glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein.

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throughout each experiment with a hydrated 95%:5% O_2:CO_2 gas mixture. The system was enclosed in a temperature-controlled, heated (37 ± 1.2°C) Plexi-glas® chamber.

Surgical Procedures

The surgical procedures used have been previously described (12). Briefly, after anesthetizing the rats with 50 mg/kg sodium pentobarbital intraperitoneally and making a midline abdominal incision, the right kidney was exposed, and the right renal artery cannulated *via* the mesenteric artery by inserting a blunt, 19 gauge, stainless-steel cannula bent at a 90° angle. The right ureter was cannulated with a 10 cm segment of polyethylene 10 (PE-10) tubing. Upon insertion of the cannula into the renal artery, the perfusion medium was allowed to flow immediately into the kidney. After rapidly excising the kidney from the abdominal cavity, the isolated kidney was immediately transferred to the temperature-controlled chamber.

Experimental Procedures and Sampling

Perfusate of known CsA or CsG concentration was prepared by the addition of known amounts of stock CsA or CsG solution to the perfusion medium approximately 90 minutes prior to experimentation. Prior to the start of each experiment, the cannulated kidney was allowed to stabilize in the perfusion chamber for approximately 15 minutes. During the stabilization and experimentation periods, renal artery perfusion pressure, corrected for the intrinsic apparatus pressure, was monitored continuously, and maintained between 80–100 mmHg by adjusting a pressure control valve (13).

Following a 5 minute stabilization period, ^{14}C-inulin (2.5 μCi) solution, 100 μl, was added to the perfusion medium, and after the equilibration period, 10 minute urine specimens were collected for a total of 110 minutes with 1.5 ml perfusate samples obtained at the midpoint of each urine collection period. The perfusate samples removed during each experiment were replaced with an equivalent volume of filtered perfusate solution. The perfusate volume removed as urine was replaced with an equivalent volume of Krebs-Henseleit buffer. All perfusate and urine samples were stored at 4°C until assayed. The perfusate and urine samples were used to assess renal function and to determine CsA or CsG concentration.

At the completion of each experiment, the kidney was perfused for 5 minutes with drug-free perfusate and weighed, and then sliced longitudinally in half. One half of each kidney was stored at −20°C until assayed for its CsA or CsG content. The other kidney half was fixed in 10% formalin and prepared for histological examination.

Perfusion Media

Each kidney was perfused with cell-free perfusion medium containing 6% protein, D-glucose (100 mg/dl), and a mixture of twenty essential L-amino acids (14) dissolved in Krebs-Henseleit bicarbonate buffer (15). Added protein consisted of BSA only (control), mixture of LDL (200 mg/dl) and BSA, or mixture of HDL (200 mg/dl) and BSA. Perfusion media containing 6% total protein was used because protein concentrations less than 5% have been shown to decrease fluid and sodium reabsorption while, higher concentrations (7.5%) resulted in lower glomerular filtration rates (13). *In vivo*, the plasma lipoprotein concentration is approximately 200 mg/dl in man (16). Therefore, perfusion media containing this concentration of lipoproteins were used to simulate lipoproteins in the plasma. The perfusion media were prepared by the addition of known amounts of LDL or HDL (200 mg/dl) to the perfusate mixture, followed by the addition of BSA to bring the total protein concentration to 6%. The pH of the final perfusion solution was adjusted to 7.4 with phosphoric acid and stored at 4 ± 1°C until used.

For each experiment, 300 ml of perfusion medium was adjusted to pH 7.4, and filtered through a 0.22 μm membrane filter immediately prior to use. The initial volume used in each experiment was 200 ml. The pH of the perfusate was maintained at 7.4 throughout each experiment by the addition of dilute H_3PO_4.

Isolation of Lipoproteins

After centrifuging 500 ml aliquots of fresh bovine blood at 20,000 *g* for 30 minutes, the serum fractions were removed and pooled. HDL and LDL were isolated and purified from the pooled serum using a modification of the method of Burstein *et al.* (17).

Isolation of Low Density Lipoprotein

To 500 ml serum samples were added 5 ml of 10% dextran sulfate (15,000 MW; Sigma Chemical Co., St. Louis, MO) solution and 25 ml of 1 M manganese chloride solution. After allowing the mixture to stand for 5–10 minutes, the precipitated LDL was separated by centrifugation at 6000 *g* at 20 ± 1.5°C for 10 minutes. The supernate of each fraction was pooled and used to isolate HDL.

The LDL isolated above was further processed as previously described (17) using twice the reported volumes of 2M magnesium chloride solution, and purified by dialysis. The purified LDL fractions were pooled and stored at 4°C until used. The pooled LDL was verified and analyzed for its lipoprotein content by gel electrophoresis using standard methods (18).

Isolation of High Density Lipoprotein

The pooled supernatant fraction that remained after the separation of LDL described above was also processed in 500 ml batches as previously described (17) to isolate HDL. The purified HDL obtained was stored at 4°C until used, and also verified and analyzed for its lipoprotein content by gel electrophoresis (18).

Renal Function Tests

The following standard tests were used to assess kidney viability, (a) glomerular filtration rate (GFR), (b) glucose excretion ($FE_{glucose}$), and (c) sodium excretion (FE_{sodium}) (19).

Total ^{14}C-inulin radioactivity in the perfusate and urine samples was determined by liquid scintillation spectrometry with external standardization (Tri-carb Liquid Scintillation Counter, Model Series 4000, Packard Instruments Co., Meriden, CT). Renal clearance of inulin was taken to represent GFR.

Perfusate and urine glucose concentrations were determined with an enzymatic method using Trinder® reagent (Sigma Chemical Co., St. Louis, MO) and colorimetric analysis (20).

Samples were analyzed at 505 nm using an UV-visible spectrophotometer (Beckman Instruments, Inc., Irvine, CA). Glucose fraction excreted in the urine ($FE_{glucose}$) was determined by $CL_{glucose}/GFR$. Perfusate and urine sodium concentrations were determined by flame atomic absorption spectrophotometry. The lower limit of sensitivity of the assay was 0.012 μg/ml (0.52 mM). The sodium fraction excreted in the urine (FE_{sodium}) was determined by CL_{sodium}/GFR. $CL_{glucose}$ and CL_{sodium} are renal clearance of glucose and sodium, respectively. Renal function was considered normal when $FE_{glucose}$ was less than 0.05 (21) and FE_{sodium} was less than 0.10–0.20 (22).

Plasma Protein Binding Studies

The interaction of CsA and CsG with plasma proteins was investigated by the ultracentrifugation method. Perfusate samples containing known concentrations of CsA or CsG were prepared over the concentration range of 150–6,000 ng/ml by adding known quantities of stock CsA or CsG solution to perfusion medium. The interaction of CsA and CsG in three different perfusion media, each containing 6% protein, was studied: (a) BSA alone (control), (b) LDL (200 mg/dl) and BSA, and (c) HDL (200 mg/dl) and BSA. The interaction of CsA and CsG with plasma proteins in each perfusion medium and concentration was determined in triplicate, and the results averaged.

Perfusate specimens of known CsA or CsG concentration were centrifuged at 180,000 *g* at 37 ± 0.5°C for 24 hours using a Beckman Model L8-70 ultracentrifuge and 50-2Ti rotor (Beckman Instruments, Inc., Palo Alto, CA) (7). After centrifugation, 600 μl of the upper, protein-free supernatant layer were carefully removed in two separate 100 and 500 μl portions for the determination of protein and unbound perfusate CsA or CsG concentrations, respectively.

Bound perfusate CsA and CsG concentrations were calculated as the difference between the total (C_T)and unbound perfusate drug (C_f) concentrations. All supernatant samples were analyzed for protein with the Bio-Rad® protein assay (Bio-Rad Laboratories, Hercules, CA) (23). The unbound fraction (f_u) of CsA or CsG in each perfusate sample was determined by the relationship, $f_u = C_f/C_T$.

Drug Assays

CsA and CsG were assayed by HPLC following solid-phase extraction using modifications of the methods of Moyer *et al.* (24).

Solid Phase Extraction

Briefly, 1 ml of 10% methanol in acetonitrile was added to 1 ml samples of perfusate, urine, or standard solution to precipitate proteins. After centrifugation, the supernate was decanted onto a C_{18} cartridge (Bond Elut®, Varian, Harbor City, CA) and washed with 2 ml each of 70% methanol and 1% hexane in acetone. CsA, CsG, and CsD, the internal standard, were eluted from the cartridge with 2 ml of 1:3 isopropanol:ethyl acetate. The effluent from the C_{18} cartridge was placed onto a silica cartridge (Bond Elut®, Varian, Harbor City, CA) from which the effluent was collected and evaporated to dryness under a nitrogen stream. For HPLC analysis, the residue was reconstituted in 100 μl of mobile phase.

The perfusate samples (500 μl) obtained in the plasma protein binding studies were extracted in the same manner with the following minor changes. The elution volume was increased to 3 ml, and the volume of wash solutions was reduced to 1 ml.

HPLC Analysis

Stock CsA and CsG solutions (100 μg/ml) were prepared in methanol and used to prepare standard solutions of CsA and CsG in concentrations ranging from 0–4,000 ng/ml.

The mobile phase (70:30 acetonitrile:water) was pumped (LDC Constametric III, Laboratory Data Control, Riviera Beach, FL) through a heated (80°C) (Fiatron Systems, Inc., Milwaukee, WI) 15 cm × 4.6 mm i.d., 5 μm C_{18} column (Altex Ultrasphere®, Beckman Instruments, Inc., San Ramon, CA) at a flow rate of 1 ml/min. The eluate was monitored at 214 nm with a variable wavelength ultraviolet detector (Model SPD-6AV, Shimadzu Scientific Instrument, Inc., Kyoto, Japan). Under these conditions, the retention times of CsA, CsG and CsD were 9.5, 12.1 and 13.2, respectively.

Standard solutions were analyzed in duplicate, and the results averaged. Standard curves, fitted to a quadratic equation, were obtained by weighted, nonlinear, least-squares regression analysis. The correlation coefficients were ≥0.99. The within-day variability (CV%) in the presence of LDL for CsA and CsG was 3.4–7.7 and 5.1–12.4%, respectively. The corresponding values for between-day variability were 1.3–11.7 and 4.2–15.8%. The within-day variability for CsA and CsG in the

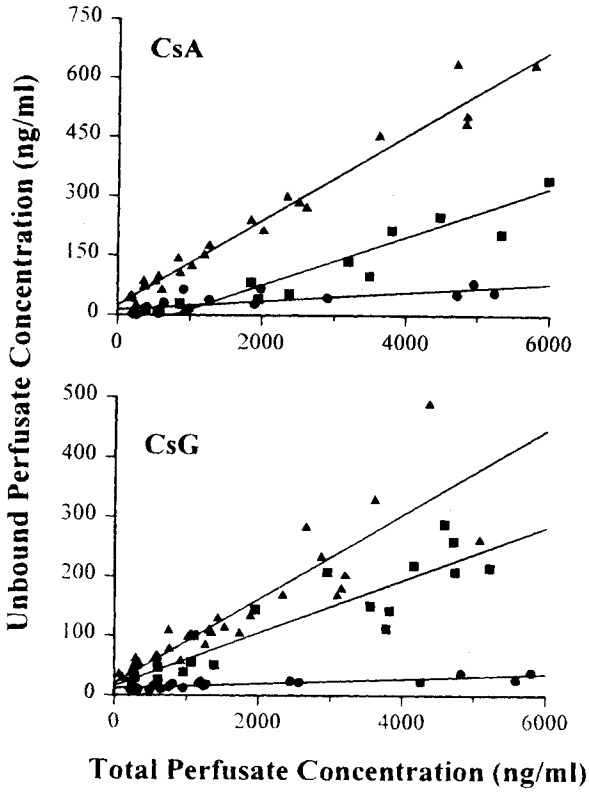

Fig. 1. Relationships between unbound perfusate CsA or CsG concentration and total perfusate CsA or CsG concentration over the range of 0 to 6,000 ng/ml in perfusion medium containing 6% BSA (▲), BSA with added LDL (200 mg/dl) (■), and BSA with added HDL (200 mg/dl) (●).

presence of HDL was 2.7–8.9 and 2.8–11.5%, respectively. The corresponding values for between-day variability were 6.3–14.4 and 6.9–14.2%. The lower limit of detection for CsA and CsG was 10 ng/ml.

Renal Tissue Accumulation

After thawing, each kidney half was separated into cortical and medullary tissue by careful dissection, and each portion weighed. After mincing, each tissue fraction was homogenized with 1 ml of distilled, deionized water using a hand-held tissue homogenizer. The tissue homogenates were extracted with 3 ml of 10% methanol in acetonitrile solution, and after mixing with a vortex mixer, the mixture was centrifuged for 10 minutes.

After separation, the supernate was removed and split into two portions, and then placed onto two Bond Elut® C_{18} cartridges. The samples were extracted as previously described, except that the effluents from the two cartridges were combined before being evaporated. After reconstituting the residue in mobile phase, tissue CsA and CsG concentrations were determined by the HPLC method.

Data Analysis

Renal clearance (CL_R) of CsA and CsG, inulin, glucose and sodium was assessed according to $CL_R = U * V/C_{mid}$, where U is the urine concentration, V is the urine flow rate, and C_{mid} is the concentration of the perfusate sample at the midpoint of the urine collection period (25).

The results were evaluated by one-way analysis of variance (ANOVA) or Students' t-test for unpaired data and a significance level of 0.05. The Newman-Keul's Multiple Range Test was used for *post hoc* analyses to determine differences between group means. Correlations were assessed by weighted, least-squares regression analysis.

RESULTS

Plasma Protein Binding Studies

As shown in Figure 1, the addition of lipoproteins to the perfusion media significantly reduced the concentrations of unbound or free CsA and CsG in the perfusate, and the effects were similar for both drugs. In perfusion medium containing 6% BSA, the mean unbound fraction (f_u) of CsA and CsG over the concentration range of 0–6,000 ng/ml was 14.7% and 11.9%, respectively. The addition of LDL (200 mg/dl) to the perfusion medium reduced f_u over the same concentration range to 3.9% and 5.9% for CsA and CsG, respectively (Table 1). The addition of HDL reduced the unbound concentrations even lower. f_u was 2.1% for CsA, and 1.8% for CsG, in perfusate containing HDL (200 mg/dl)(Table 1).

Table 1. Mean ± S.D. Unbound Fraction of CsA and CsG in Perfusate Containing 6% Total Protein as BSA, LDL (200 mg/dl) and BSA and HDL (200 mg/dl) and BSA

	Unbound fraction, f_u (%)		
Drug	BSA	LDL and BSA	HDL and BSA
CsA	14.7 ± 5.0	3.9 ± 1.6[a]	2.1 ± 1.6[a,b]
CsG	11.9 ± 8.9	5.9 ± 2.3[a,c]	1.8 ± 1.0[a,b,c]

[a] Significantly different from corresponding BSA data, $p < 0.05$.
[b] Significantly different from corresponding LDL data, $p < 0.05$.
[c] Significantly different from corresponding CsA data, $p < 0.05$.

Renal Excretion Studies

The results of the renal CsA and CsG excretion studies are summarized in Figure 2. As shown in Figure 2, renal excretion of CsA and CsG was significantly diminished by the addition of LDL and HDL to the perfusion medium. With perfusate containing 6% BSA only, the mean renal clearance (CL_R) of CsA and CsG was 0.024 and 0.022 ml/min, respectively. The addition of LDL or HDL (200 mg/dl) to the perfusion medium resulted in a lowering of CL_R for CsA to 0.007 and 0.006 ml/min with LDL and HDL, respectively, and 0.005 and 0.007 ml/min for CsG (Table 2).

Renal Tissue Distribution

Table 3 summarizes the renal tissue concentrations of CsA and CsG observed in this investigation. Although the results for the individual tissue concentrations were highly variable, when compared to kidneys perfused with 6% BSA alone in the perfusion medium, whole organ and cortical and medullary tissue CsA and CsG concentrations were several-fold lower in kidneys perfused with media containing LDL and HDL (200

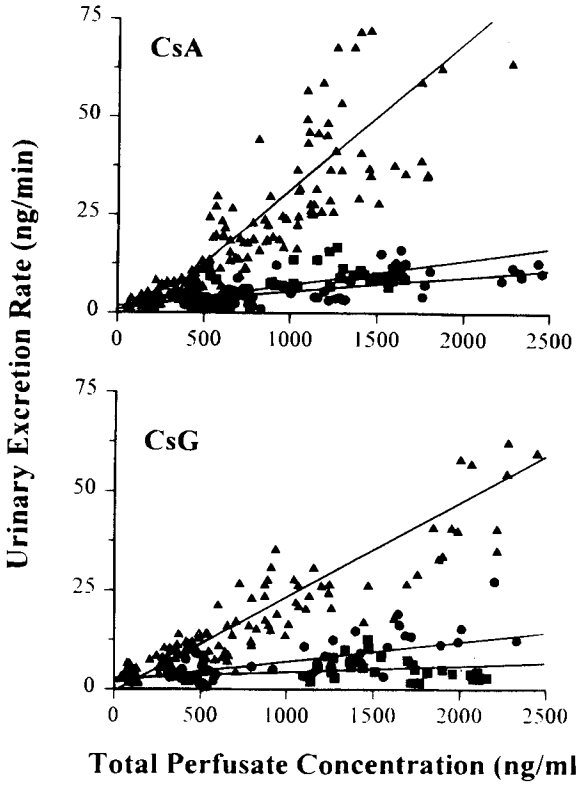

Fig. 2. Relationships between urinary CsA or CsG excretion rate and total perfusate concentration with perfusion medium containing 6% BSA (▲), BSA with added LDL (200 mg/dl) (■), and BSA with added HDL (200 mg/dl) (●). Each datum point represents an individual renal CsA or CsG clearance determination. The solid lines are the best-fit lines for the data.

Table 2. Mean S.D. Renal CsA and CsG Clearance Observed with Perfusate Containing 6% Total Protein as BSA, LDL (200 mg/dl) and BSA, and HDL (200 mg/dl) and BSA

	Renal clearance, CL_R (ml/min)		
Drug	BSA	LDL and BSA	HDL and BSA
CsA	0.024 ± 0.010	0.007 ± 0.004[a]	0.006 ± 0.003[a]
CsG	0.022 ± 0.007	0.005 ± 0.002[a,b]	0.007 ± 0.004[a]

[a] Significantly different from corresponding BSA data, $p < 0.05$.
[b] Significantly different from corresponding CSA data, $p < 0.05$.

mg/dl). These observations were consistent with the results obtained in the protein binding studies.

The effects of LDL and HDL (200 mg/dl) on renal CsA distribution were similar (Table 3). With CsG, on the other hand, the data suggested that HDL had a greater effect than LDL in reducing CsG distribution into renal tissues.

In this study, for a given treatment, no differences in CsA or CsG concentrations were observed for either drug between cortical and corresponding medullary tissues.

Renal Function Studies

Renal function tests were performed to assess the viability of the kidney preparation during each experiment and to monitor for potential drug effects on renal function (Table 4). In this study, $FE_{glucose}$ was within the normal ranges in all kidney experiments. GFR for CsA- and CsG-treated kidneys was higher than control kidneys perfused with LDL (200 mg/dl), and similar to corresponding controls perfused with HDL (200 mg/dl). Sodium excretion in kidneys perfused with LDL (200 mg/dl) was less than the corresponding controls in kidneys perfused with CsA, but was not different when perfused with CsG. FE_{sodium} was greater than those of the controls in kidneys perfused with CsA or CsG in perfusion medium containing HDL (200 mg/dl).

DISCUSSION

This study demonstrated the effects of lipoproteins on drug disposition. The interactions of CsA and CsG with LDL and HDL significantly reduced the concentrations of unbound or free drug in the perfusion media, and the effects were similar for both compounds. f_u for CsG and CsA was diminished two- and four-fold, respectively, in the presence of LDL and approximately seven-fold in the presence of HDL. These observations were consistent with previous investigations in which it was found that in human plasma, CsA was bound primarily to lipoproteins (4) and that the affinity was greatest for HDL (6).

In this study, the interaction of CsA and CsG with plasma proteins in the three perfusion media was concentration-independent over the range of 0–6,000 ng/ml. This finding was in agreement with previous studies with CsA where the extent of CsA binding to plasma proteins was concentration-invariant with plasma CsA concentrations ranging up to 20,000 ng/ml (4,5,7).

Of note, the unbound fractions (f_u) of CsA and CsG observed in the present investigation in *in vitro* perfusion fluids were similar to those reported for CsA in human plasma. Niederberger *et al.* (5) reported an f_u for CsA of approximately 2%. In renal transplant patients, f_u values for CsA of 4.2 to 12% (7), and 7% have been observed (8).

As presented in Figure 2 and Table 2, CL_R of CsA and CsG were diminished approximately four-fold each in the presence of LDL and HDL. Since renal drug excretion is dependent on the unbound drug concentrations, these observations for CL_R for CsA and CsG in the presence of lipoproteins were in agreement with the observed reductions in f_u seen in the *in vitro* binding studies.

Since only unbound drug is capable of distributing out of the vascular system into surrounding tissues, the interaction of drugs with plasma proteins will effect how drugs distribute in the body. In the present investigation, when compared to controls, renal tissue CsA and CsG concentrations were significantly lower in the kidneys perfused with perfusion media containing LDL and HDL (Table 3). These observations could be attributed to an increase in protein binding of CsA and CsG to the lipoproteins in the perfusion medium. Moreover, the lower tissue CsA and CsG concentrations were consistent with the histological observations described below. A similar effect of LDL on CsA distribution was seen when studied using hepatic cell lines (26). A 60% reduction in cellular CsA uptake was observed when the cells were bathed in media containing LDL (compared to LDL-free media), CsA uptake decreasing as LDL concentrations increased.

In the same manner, by altering the pattern of drug distribution, lipoproteins may also influence the toxicity of drugs. Patients with high serum CsA concentrations associated with hypertriglyceridemia and hypercholesterolemia exhibited no signs of CsA toxicity (27). In contrast, patients with normal serum CsA concentrations with hypocholesterolemia showed signs of increased toxicity (28). Similar observations were noted in the present investigation. Kidneys perfused with CsA and CsG in the perfusion medium containing 6% BSA alone exhibited inflammation, edema and microvacuolization on histological examination, signs of CsA toxicity. In kidneys perfused with perfusion media containing LDL or HDL, on the other hand, no histological changes were observed.

The elevated FE_{sodium} observed in kidneys perfused with CsA and CsG in perfusion medium containing HDL (200 mg/dl) can be attributed to an effect of the drugs. However, the observed lower FE_{sodium} in kidneys perfused with CsA in perfusate containing LDL and increased GFR with both drugs (compared to the controls) could not be explained.

Clinically, the interaction of CsA and CsG with LDL and HDL observed in the present investigation are significant

Table 3. Mean ± S.D. Renal Tissue CsA and CsG Concentrations Obtained in Kidneys Perfused with Perfusate Containing 6% Total Protein as BSA, LDL (200 mg/dl) and BSA, and HDL (200 mg/dl) and BSA

	Renal tissue concentration (μg/g)		
Drug	BSA	LDL and BSA	HDL and BSA
CsA	33.9 ± 23.4	9.5 ± 6.1[a]	5.3 ± 1.8[a]
CsG	21.5 ± 19.9	7.2 ± 6.5[a]	2.0 ± 1.0[a,b]

[a] Significantly different from corresponding BSA data, $p < 0.05$.
[b] Significantly different from corresponding CsA data, $p < 0.05$.

Table 4. Mean ± S.D. Viability Parameters for Control (No Drug), CsA, and CsG Perfused Kidneys Using Perfusate Containing 6% Protein as BSA, LDL (200 mg/dl) and BSA, and HDL (200 mg/dl) and BSA

Treatment	GFR (ml/min)	$FE_{glucose}$	FE_{sodium}	Urine flow rate (ml/min)
BSA				
Control	0.91 ± 0.17	0.034 ± 0.020	0.12 ± 0.06	0.13 ± 0.03
CsA	0.69 ± 0.25[a]	0.030 ± 0.031	0.20 ± 0.10[a]	0.15 ± 0.04[a]
CsG	0.74 ± 0.21[a]	0.025 ± 0.018[a]	0.22 ± 0.10[a]	0.18 ± 0.04[a]
LDL				
Control	0.23 ± 0.12	0.030 ± 0.021	0.22 ± 0.10	0.06 ± 0.02
CsA	0.46 ± 0.18[a]	0.036 ± 0.017	0.12 ± 0.06[a]	0.07 ± 0.02
CsG	0.30 ± 0.15[a]	0.028 ± 0.021	0.19 ± 0.08	0.07 ± 0.03
HDL				
Control	0.59 ± 0.23	0.029 ± 0.019	0.10 ± 0.05	0.07 ± 0.03
CsA	0.54 ± 0.16	0.039 ± 0.019[a]	0.22 ± 0.10[a]	0.13 ± 0.03[a]
CsG	0.55 ± 0.17	0.036 ± 0.024	0.18 ± 0.09[a]	0.12 ± 0.04[a]

[a] Significantly different from control data, $p < 0.05$.

because of their potential to influence or alter drug disposition and/or toxicity (9). Patients receiving CsA reportedly experience elevated plasma cholesterol levels at 90 days post-organ transplantation (11). In that study, plasma LDL concentrations were elevated in all patients. HDL and VLDL levels were also increased, but to lesser extents. In another study (29), elevated LDL and VLDL concentrations were observed after CsA treatment. Chronic CsA administration has been also associated with elevated plasma cholesterol concentrations (i.e., LDL and HDL) in bone marrow transplant patients and patients with autoimmune disease (30).

The results of this investigation demonstrated that LDL and HDL significantly altered the disposition characteristics of CsA and CsG in the isolated perfused rat kidney. These data suggested that monitoring plasma lipoprotein concentrations in patients receiving CsA may be an important factor to consider when using the drug.

ACKNOWLEDGMENTS

The authors thank Drs. David Smith and Alan Kugler for their assistance in establishing the isolated perfused rat kidney model in our laboratory, Dr. James Wisecarver for his assistance with the evaluation of the histological specimens, Emily J. Moore for her technical assistance, the Sandoz Pharmaceutical Corporation for the cyclosporine A, cyclosporine D, and cyclosporine G powder, the Pharmaceutics Research Fund for support of this study, and the Greater Omaha Packing Co., Omaha, NE for the fresh bovine blood from which the lipoprotein fractions were isolated.

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