Insights Into the Effects of Hyperlipoproteinemia on Cyclosporine A Biodistribution and Relationship to Renal Function

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Abstract

The purpose of this study was to assess the effect of hyperlipoproteinemia on the biodistribution of cyclosporine A (CyA), an extensively lipoprotein bound immunosuppressant, in a rat model and to determine the potential toxicological significance of this effect. Normolipidemic and hyperlipoproteinemic rats were given a single 5 mg/kg dose of CyA as intravenous bolus and at selected times postdose, tissues, blood, and plasma were harvested and assayed for CyA content. Hyperlipoproteinemia was induced by intraperitoneal injection of 1 g/kg poloxamer 407. Compared with normolipidemic animals, hyperlipoproteinemic rats had higher plasma, blood, kidney, and liver CyA concentrations. In contrast, in heart and spleen the concentrations were decreased in hyperlipoproteinemia. The nephrotoxic effect of CyA was also evaluated in normolipidemic and hyperlipoproteinemic rats after 7 days of dosing with 20 mg/kg/day. In both groups of animals, repeated doses of CyA were associated with equivalent decreases in creatinine and urea clearances compared with matching control and predose baseline measures. The concentrations of drug in kidney were equivalent at the conclusion of the study. However, despite these similarities there was microscopic evidence of more severe changes in the kidneys in the hyperlipoproteinemic rats, which also experienced a significant decrease in body weight compared with the normolipedemic animals. In conclusion, the distribution of CyA to kidneys was enhanced in poloxamer 407–induced hyperlipoproteinemic rats after single doses, and with repeated doses there was an apparent greater adverse effect on these animals compared with normolipidemic animals.

Keywords: Biodistribution, hyperlipoproteinemia, protein binding, nephrotoxicity

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Introduction

Cyclosporine A (CyA) is a lipophilic cyclic endecapeptide originally derived from the filamentous fungus *Tolypocladium inflatum*.[1] Its introduction to the market was a major advance in immunosuppression and led to significant improvements in the outcomes of organ transplantation.[2] The major dose-limiting side effect of CyA is nephrotoxicity.[3] Functionally, this in part takes the form of a decrease in creatinine clearance (CLcr).[4] Although the exact cause is not known, evidence is available implicating the activation of the renin-angiotensin system[5] and renal vasoconstriction.[6] This could cause ischemia and production of reactive oxygen species,[7-9] which could disrupt renal cellular function. Oxidative stress,[10] nitric oxide generation,[11] elevated P53 levels with apoptosis,[12] and vascular endothelial growth factor[13] are all thought to be involved in CyA-associated renal toxicity. CyA is also associated with a highly variable pharmacokinetic profile. Because the drug has a narrow therapeutic range of blood concentrations, from a clinical management point of view this variability can be highly problematic in selection of an optimal dosage regimen for an individual patient. Understanding the sources of variation in drug disposition can assist in devising dosing strategies that can provide a more consistent and predictable range of blood concentrations.

CyA is extensively distributed to tissues, which is reflected in its high apparent volume of distribution (Vd). High concentrations of CyA have been reported in leukocyte- and fat-rich organs.[14] CyA is almost entirely metabolized in the liver, and therefore its total body clearance (CL) is very close to that of its hepatic CL. In the systemic circulation, CyA is bound ~50% to red blood cells, 15% to leukocytes, and 25% to plasma proteins.[15] The unbound fraction of CyA present in the plasma is typically less than 10% in humans,[16] and up to 20% to 30% in rats.[17]

Lipoproteins are a heterogeneous population of macromolecular aggregates composed of lipids and proteins and are primarily responsible for the transport of lipids through the vascular and extravascular fluids. They may also act as carriers of several hydrophobic drugs within the circulation and are the major protein class involved in the binding of CyA in plasma.[18] In normolipidemic (NL) human plasma, 31% of CyA is associated with combined low-density lipoprotein (LDL) and very low density lipoprotein (VLDL)

classes, 44% with high-density lipoprotein (HDL), and 20% with other protein classes such as albumin.[19] In hyperlipoproteinemic (HL) human plasma there is a shift of drug into the LDL/VLDL fractions from the HDL fraction.[19]

In the presence of elevated lipoprotein plasma concentrations as a result of a high-fat meal, or in experimental models of HL, the pharmacokinetics of CyA do not always respond as expected. Some studies have reported either no change or an increase in plasma or blood CL rather than the expected reduction in total body CL.[17,20,21] It has been proposed that in the presence of HL, a lipoprotein-associated increase in the sequestration of CyA by tissues rich in LDL receptors, such as the liver, is responsible for the findings.[20,21]

To better understand the effects of HL on CyA pharmacokinetics and toxicodynamics, in this report we describe the biodistribution pattern and the extent of nephrotoxicity of CyA in the presence and absence of an experimentally induced HL state caused by intraperitoneal (ip) injection of poloxamer 407 (P407) to rats.[22,23] By examining the concentrations of CyA in the tissues, particularly the liver, we hoped to shed some light on the observed lack of decrease in CyA CL given the previously demonstrated decrease in unbound fraction in plasma.[17] Based on previous findings, it was hypothesized that HL would cause a change in the biodistribution of CyA, with possible alterations in the pharmacological or toxicological potency of the drug.

Materials and Methods

Chemicals

CyA was used as Sandimmune 50 mg/mL for intravenous (IV) administration (Novartis, Dorval, PQ). Both CyA and heparin (Leo Pharma, Thornhill, ON) were purchased from the University of Alberta Hospital Pharmacy. P407 and amiodarone HCl (the internal standard for assay of CyA) were supplied by Sigma (St Louis, MO). Halothane was purchased from MTC Pharmaceuticals (Cambridge, ON). Sodium chloride injection 0.9% was obtained from Abbott Laboratories (Montreal, PQ). All other chemicals were purchased from Fisher Scientific (Nepean, ON).

Animals

The experimental protocols were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. All animal experiments were performed using male Sprague-Dawley rats (Charles River Laboratories, Montreal, PQ) with body weights of 311 ± 26.5 g (range 260-350 g) for the biodistribution study, and 250 ± 19.4 g (range 215-279 g) for the nephrotoxicity experiments. All rats were housed in a temperature-controlled room with a 12-hour light/dark cycle for at least 1 week before the experiments and during the nephrotoxicity study. The animals had free access to water and a standard rodent chow containing 4.5% fat (LabDiet 5001, PMI Nutrition LLC, Richmond, IN) prior to and during the experiments.

Biodistribution Study

HL was induced in the rats by ip administration of 1 g/kg P407 as a 0.13 g/mL solution in cold normal saline. The dose was injected 36 hours before the biodistribution study under light halothane anesthesia. A small blood sample was withdrawn 24 hours after each injection and examined visually for appearance of lipemic plasma, to ensure the proper administration of P407.

Rats were randomly assigned to NL or HL groups; there was no significant difference between rats in either group. Under light halothane anesthesia, each rat received 5 mg/kg CyA diluted 10-fold in sterile 0.9% NaCl as IV bolus injection through the tail vein. At 5 minutes after dosing, while left under anesthesia, some rats were exsanguinated by cardiac puncture, and the heart, spleen, liver, kidney, fat, brain, and whole blood samples were collected. In the remaining rats studied, the animals were allowed to recover from anesthesia after administration of CyA. At 1, 2, 6, 12, or 24 hours after drug injection the animals were again anesthetized and the specimens were collected as they had been for the 5-minute rats. At each time point, 4 rats were included. To separate the plasma, blood samples were centrifuged at 2500 g for 5 minutes and the supernatant plasma was saved for analysis. After extraction, tissues were blotted with tissue paper to remove excess blood. Specimens were stored in phosphate buffer (pH = 7.4) at –30°C until they were assayed for drug concentration by high-performance liquid chromatography.

Nephrotoxicity Experiments

The nephrotoxic effects of repeated doses of CyA were assessed in NL and HL rats. Before each experiment, a baseline 24-hour urine sample (collected in a metabolic cage) and a blood sample (150-200 μL) taken during the urine collection interval were collected from each rat to assess the baseline renal function prior to the treatment period. The day before the experiments, the right jugular vein of each rat was catheterized with Silastic tubing (Midland, MI) under halothane/O_2, administered by anesthetic machine. The cannula was flushed and filled with 100 U/mL heparin in 0.9% saline. In the HL groups, the initial dose of P407 was injected immediately after cannula implantation. After surgery, the rats were transferred to regular holding cages

and allowed free access to water and food. Animals were weighed each day at 0730 hours.

Because of the highly variable and unpredictable pharmacokinetics and bioavailability of oral CyA, IV injection was chosen as the route of administration in the nephrotoxicity experiments. The dose was based on previous repeat-dose studies where a drug was given by the subcutaneous or ip routes,[24,25] taking into account the bioavailability by those routes. To maximize drug accumulation, the daily dose was divided into 3 equal doses. Animals were divided into 4 groups with 5 to 6 rats in each group. NL or HL rats received either normal saline (control groups) or 20 mg CyA/kg/day × 7 days as IV administration. All doses were infused over 60 seconds through the cannula, 3 times a day (6.67 mg/kg/dose). Cannulas were filled with diluted heparin (100 U/mL normal saline) after each injection to prevent blood coagulation inside the cannula.

To sustain the HL during the nephrotoxicity experiments, the ip injection of P407 was repeated every 72 hours from the time of the initial P407 dose. Therefore, a total of 3 P407 doses were administered, the first ~18 hours before the experiment began, and the second and third on days 2 and 5 after the IV dosing of saline or CyA had commenced.

On the last full day of treatment, rats were transferred to metabolic cages and a 24-hour urine collection was conducted. Three hours after the last dose of CyA had been administered, the animals were anesthetized and exsanguinated by cardiac puncture to collect blood and kidney samples. These specimens were frozen at –30ºC until they were assayed for CyA.

Analytical Methods

A validated method for analysis was used to assay CyA in the specimens.[17,26,27] For construction of standard curves, drug-free samples from NL or HL animals were used to quantify CyA in drug-treated NL or HL animals, respectively. Standard curves yielded r^2 of >0.99 in all analyses for CyA. Calibration curves yielded excellent linearity in peak height ratio of drug-to-internal-standard vs drug concentration in plasma, blood, and tissue homogenates.[17,26] Renal function was assessed by determination of creatinine and urea levels in urine samples, and creatinine, sodium, potassium, and urea levels in plasma samples, using a Synchron LX system (Beckman Coulter, Fullerton, CA). All analyses were performed by the University of Alberta Hospitals Department of Laboratory Medicine and Pathology (accredited by the College of American Pathologists). Because lipemic plasma can interfere with some clinical chemistry analysis, for determination of creatinine the HL plasma samples were first ultracentrifuged to separate the lipids from plasma. Lipoclear (Iris Sample Processing, Westwood, MA) was used to separate lipoproteins prior to analysis for plasma electrolytes.

Creatinine and urea CLs were estimated using the following equation:

$$Clearance = \frac{Urine\ concentration \times urine\ volume}{Serum\ concentration} \quad (1)$$

Kits for measurement of triglyceride and total cholesterol were purchased from Diagnostic Chemicals Limited (Charlottetown, PE). The methods involved colorimetric detection based on oxidation of free cholesterol and hydrolysis of cholesterol esters and triglycerides. The test was performed according to the instructions provided by the manufacturer.

Renal Histological Studies

The kidneys were washed with ice-cold saline after extraction. For histological examination, the organ was fixed in 10% neutral buffered formalin solution and embedded in paraffin. Slices of 2-μm thickness were cut, deparaffinized, hydrated, and stained with hematoxylin-eosin. An additional section of each kidney was stained with periodic acid–Schiff (PAS). Sections were examined visually (magnification power 100-400×) for apical blebbing, interstitial vacuolization, hyaline casts, glomerular changes, arteriolopathy, and tubulointerstitial fibrosis in CyA-treated groups.

Each slide was assigned a code number before being sent off for microscopic assessment. The microscopic assessments were performed by a qualified veterinary pathologist, Dr Nick Nation, DVM, from the University of Alberta, Health Sciences Laboratory Animal Services. The assessor, who was blinded to the treatment groups and to the nature of the study design, was asked to look for qualitative evidence of microscopic changes consistent with renal toxicity. The sections (1 per kidney per animal) were each ~0.5 to 1 cm in diameter. Numerous fields were examined for evidence of consistent changes within the affixed kidney section.

Data and Statistical Analysis

Unless otherwise stated, all compiled data are presented below as mean ± SD. The area under the CyA concentration vs time curve (AUC) based on the mean data from each sampling time point were calculated for each of the tissues and for blood and plasma. In the tissue distribution studies, because of the destructive study design, AUC could not be determined for individual rats in each group. For this purpose the Bailer method[28] was used. In this test, α was 0.05, the critical value of Z (Z_{crit}) for the 2-sided test after Bonferroni adjustment was 2.24, and the observed value of Z (Z_{obs}) was calculated as previously described.[26,28] The tissue-to-plasma (kp) or -blood (kb) concentration ratios were also determined for AUC or for postdistributive phase samples.

In comparing physiological factors related to renal function in the nephrotoxicity study, Student t test for paired or unpaired data, as appropriate, was used to assess the significance of differences. The level of significance was set at $P = .05$. Changes in body weight were assessed on day 7 in the nephrotoxicity experiments and ranked using analysis of variance followed by a post hoc Duncan's multiple range test, using SPSS for Windows v13 (Cary, NC).

RESULTS

Tissue Distribution of CyA After Single IV Doses

In the plasma of NL rats, the total triglyceride and cholesterol concentrations were 0.833 ± 0.233 and 1.13 ± 0.300 mM, respectively. In contrast, in the plasma of P407-treated rats, the total triglyceride (48.3 ± 8.47 mM) and cholesterol (27.9 ± 5.76 mM) concentrations were substantially and significantly higher. After single-dose IV CyA administration, the plasma and blood concentrations declined rapidly for 2 hours, after which the postdistributive phase appeared to be reached (Figure 1). In the tissues analyzed for drug content, CyA was detected in all specimens at quantifiable concentrations except in the brain and fat specimens. In those specimens in which CyA was detected, the order in mean AUC from highest to lowest in the NL group was spleen > liver > kidney > heart > blood > plasma. In the HL group the corresponding order was liver > kidney > spleen > plasma > heart > blood (Table 1, Figure 1). For each specimen, the mean $AUC_{0\text{-}24h}$ was significantly different for the NL and the HL animals. Compared with NL rats, in HL rats the AUC of CyA in plasma, blood, kidney, and liver was increased 2.6-, 1.5-, 1.5-, and 1.6-fold, respectively. On the other hand, the AUC of CyA was decreased in heart and spleen by 1.5- and 1.7-fold, respectively (Table 1, Figure 1).

In the HL animals, the kp and kb based on CyA AUC were either approximately the same as or lower than what they had been in the NL animals (Table 1). Although the mean value is reported, statistical testing could not be performed on the differences in kb or kp based on AUC data because of the study design (1 timed sample per animal). During the postdistributive phase of the concentration vs time curves, which occurs at ~2 hours for CyA,[17] a constant ratio is expected between concentrations of drug in tissue and plasma or blood. Therefore, when the mean postdistributive kp (ie, 2 hours postdose or more) for each tissue sample was compared (Figure 2), a significantly higher kp was noted for NL than for HL animals in all specimens except liver, where $P = .051$. For postdistributive kb the same trend was observed for heart and spleen, with NL having a significantly higher kb than HL. However, the kb of liver from HL animals was significantly higher than the kb of liver from NL animals, and for kidney no significant difference was noted between the 2 groups.

Nephrotoxicity Experiment

Similar to what was observed in the biodistribution study, 3 doses of P407 administered over 8 days caused substantial increases in the triglyceride and total cholesterol levels (Table 2). There were no significant differences in plasma cholesterol or triglyceride detected between P407-treated animals given CyA and those not given CyA. Unfortunately, there was insufficient plasma from the saline-treated NL animals to permit comparison with the saline-treated HL animals (Table 2). In HL animals the triglyceride and total cholesterol plasma concentrations after 7 days of CyA treatment and 3 doses of P407 (Table 2) did not significantly differ from those levels measured after a single dose of P407 in the biodistribution study. There was also no difference in these levels between NL animals given a single dose of CyA (biodistribution study) and those measured after 7 days of treatment with CyA (Table 2).

In the baseline (day 0) measures of renal function there were no differences between animals in any of the 4 groups (Table 2). On day 8, no significant changes were discerned between the NL and HL saline-treated animals in the indicators of renal function. Likewise, in comparing changes within animals, there were no significant differences in saline-treated NL or HL rats in their day 8 vs day 0 renal function indexes, except for plasma sodium in the HL animals. However, in the NL animals receiving CyA, significant decreases from baseline (day 0) were found in the creatinine (48%) and urea (63%) CLs. This was accompanied by a significant increase of 21% in the plasma potassium.

As in the NL animals given CyA for 7 days, in the HL rats receiving CyA, urea and creatinine CLs were significantly decreased from baseline (day 0) values, by 57% and 56%, respectively. Potassium levels were also significantly increased, by 16%. Plasma sodium was also significantly decreased in these CyA-treated HL animals. The decreases in the CLs of urea and creatinine in the animals receiving CyA in the NL group were not significantly different from those of the animals receiving CyA in the HL group.

Histopathological examination of the kidneys was performed in 4 of the CyA-treated NL rats and all of the CyA-treated HL rats. Kidney abnormalities (when kidneys were compared with normal rat kidneys) were present in both NL and HL rats, although the changes were somewhat different in each of these groups (Figure 3). Both NL and HL groups had tubular epithelial cell changes. For NL rats these presented as finely vacuolated cytoplasm (Figures 3-1 and 3-2). In contrast, the HL rats had vacuolated renal tubular epithelial cytoplasm that also contained PAS-positive and acidophilic droplets within the vacuoles (Figures 3-3 and 3-4). Glomerular defects were noted in only 1 of the kidneys from the NL animals given CyA and presented as finely vacuolated cytoplasm. The glomeruli of HL rats presented

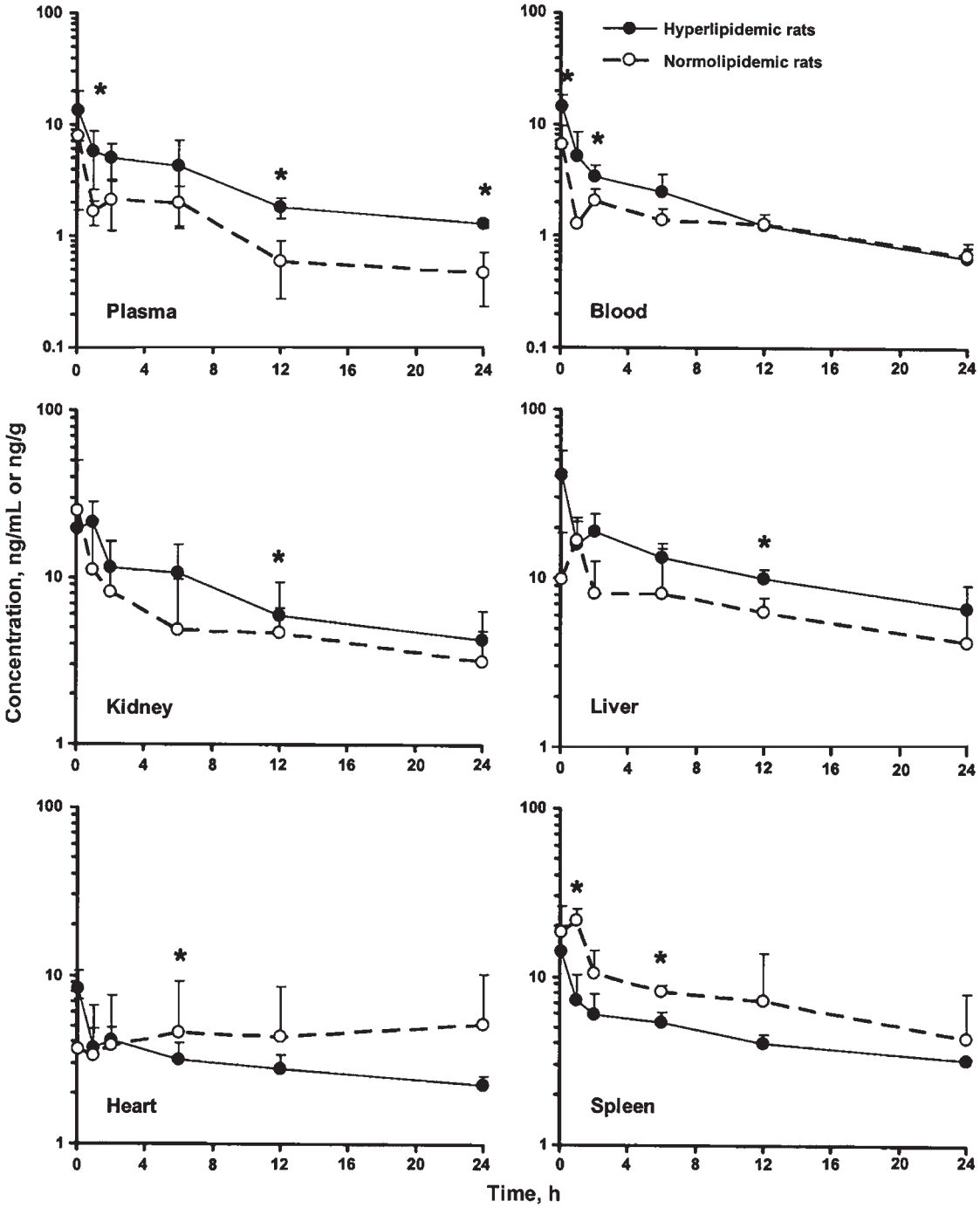

Figure 1. Cyclosporine A concentration vs time profiles in various specimens following intravenous administration of a single dose of 5 mg/kg to normolipidemic and hyperlipoproteinemic rats. * indicates significant difference between mean concentrations at the specified time point ($P < .05$).

with these types of defects more often than the glomeruli of NL rats did.

The CyA levels in the whole blood samples of the HL rats at the end of the treatment period were significantly (66.9%) higher than those of the NL rats (Figure 4). However, despite these higher blood levels, the CyA levels in the kidneys did not significantly differ between NL and HL rats (Figure 4). After 7 days of CyA administration (Figure 4), the kb of CyA in the kidneys was significantly higher (2.69 ± 1.37) in the NL than the HL group (1.79 ± 0.236). The corresponding kb of kidneys in the postdistributive phase (2-24 hours postdose) of NL rats after a single dose was 4.16 ± 1.48 (Figure 2) and was not significantly different from the values after 7 days of repeated dose administration (Figure 4). In HL rats, however, there was a significantly lower kb for kidneys after 7 days of 20 mg/kg/d compared with the kb for kidneys in rats given a single dose of 5 mg/kg (5.01 ± 1.92; Figure 2).

Table 1. Mean ± SD of AUC and kp or kb of CyA After Single 5 mg/kg Doses Intravenously to NL and HL Rats*

Parameter	Plasma	Blood	Kidney	Liver	Heart	Spleen
			NL			
AUC_{0-24} (mg-h/L)	29.5 ± 3.23	31.8 ± 1.59	128 ± 7.12	176 ± 27.5	107 ± 13.8	188 ± 32.1
kb	—	—	4.03	5.53	3.36	5.91
kp	—	—	4.34	5.97	3.63	6.37
			HL			
AUC_{0-24} (mg-h/L)	77.3 ± 8.47†	48.7 ± 3.91†	189 ± 17.3†	276 ± 13.8†	71.8 ± 3.60†	110 ± 4.59†
kb	—	—	3.88	5.66	1.47	2.25
kp	—	—	2.45	3.57	0.93	1.42
Mean HL:NL AUC ratio	2.62	1.53	1.48	1.57	0.671	0.585

*AUC indicates area under the CyA concentration vs time curve; kp, tissue-to-plasma concentration ratio; kb, tissue-to-blood concentration ratio; CyA, cyclosporine A; NL, normolipidemic; HL, hyperlipoproteinemic.
†Denotes significant difference *(P* < .05) from NL animals.

There were no significant differences between the body weights of the animals in each group at the commencement of the experiments. Mean body weights increased significantly in both the saline-treated NL and HL groups over the 7-day test period. No significant differences were noted between HL and NL rats in mean body weights at the conclusion of the study in the saline-treated animals (Figure 5). However, when CyA was administered for 7 days, there was deviation from that observed in the saline-treated animals, which translated into significant differences between the groups. In terms of relative weight gain from day 1, the rank order as determined by the Duncan's multiple range test was [saline NL = saline HL] > [CyA NL] > [CyA HL].

DISCUSSION

The ip injection of P407 causes a profound increase in circulating lipoproteins by decreasing lipoprotein hepatic lipase and increasing lecithin cholesterol acyl transferase and cholesteryl ester transfer protein activities.[29] Previously it was observed that the plasma unbound fraction of CyA, and blood Vd and terminal half-life, were reduced significantly in P407-treated rats.[17] Similar results were seen here in that mean blood concentrations were higher in the HL animals for the first 6 hours after dosing, after which they were superimposed on those of the NL animals. At half of the available time points, including the later time points of 12 and 24 hours, plasma concentrations of HL rats were significantly higher than those of NL animals. Indeed, the

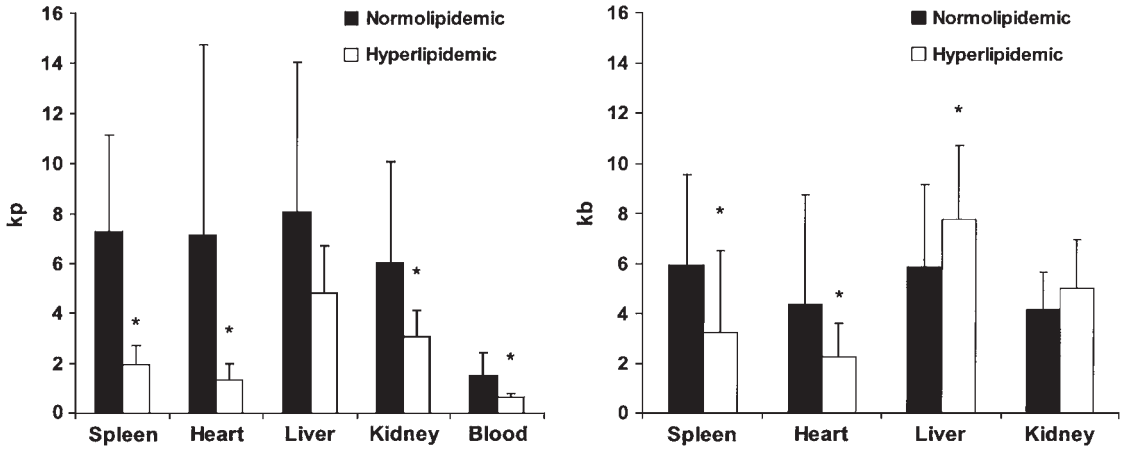

Figure 2. kp and kb of postdistributive (2-24 hours) concentrations. * indicates significant difference (*P* < .05) between hyperlipoproteinemic and normolipidemic treated animals. kp indicates tissue-to-plasma concentration ratio; kb, tissue-to-blood concentration ratio.

Table 2. Indexes of Renal Function in NL and HL Rats Before (Day 0) and After 7 Days of (Day 8) Administration of Saline (Control Groups) or CyA*

Group	Day	Plasma Na	Plasma K	Urea Clearance (mL/min/kg)	CLcr (mL/min/kg)	Plasma Triglyceride (mM)	Plasma Total Cholesterol (mM)
Saline-treated NL rats	0	141.3 ± 2.10	4.50 ± 0.59	5.74 ± 1.41	7.95 ± 1.13	—	—
Saline-treated NL rats	8	141.3 ± 1.70	4.60 ± 0.48	5.21 ± 0.89	8.50 ± 1.17	ND	ND
Saline-treated HL rats	0	142.2 ± 0.45	4.14 ± 0.15	5.69 ± 0.44	9.88 ± 0.70	—	—
Saline-treated HL rats	8	135.8 ± 3.54†	4.26 ± 0.30	5.35 ± 0.92	9.20 ± 0.69	52.2 ± 10.3	25.3 ± 4.88
CyA-treated NL rats	0	142.0 ± 1.60	3.92 ± 0.29	5.78 ± 1.55	10.23 ± 2.48	—	—
CyA-treated NL rats	8	146.0 ± 4.50	4.74 ± 0.35†	2.14 ± 0.14†	5.35 ± 1.51†	0.791 ± 254	1.19 ± 0.455
CyA-treated HL rats	0	142.5 ± 2.51	4.27 ± 0.54	6.81 ± 1.43	9.05 ± 0.79	—	—
CyA-treated HL rats	8	138.0 ± 2.02†	4.96 ± 0.29†	2.91 ± 1.73†	3.97 ± 1.59†	51.0 ± 11.3	26.5 ± 10.6

*NL indicates normolipidemic; HL, hyperlipoproteinemic; CyA, cyclosporine A; CLcr, creatinine clearance; ND, not determined.
†Significant difference compared with day 0 ($P < .05$).

blood-to-plasma concentration ratio in the postdistributive phase was higher in NL than in HL animals (Figure 2). This indicates that there was a shift in blood concentrations from erythrocytes and other blood cells, which are known to bind CyA, to the plasma portion of blood, which was enriched with high levels of circulating lipoprotein.

There was a significantly higher $AUC_{0\text{-}24h}$ in both blood and plasma in the HL rats. This is expected because of the large increase in lipoprotein levels, resulting in an increased binding of drug in plasma and a decrease in the unbound fraction in plasma. This would in turn be expected to limit the distribution of drug to peripheral tissues and possibly to decrease CL, especially for a drug with a low hepatic extraction ratio such as CyA. Only $AUC_{0\text{-}24h}$ in the biodistribution study could be calculated, because individual estimates of half-life were not available. However, the apparent terminal phase half-life based on the mean blood concentration data suggested a shorter value for the HL animals. If extrapolated to infinity, the resultant $AUC_{0\text{-}\infty}$, if used to calculate CL (ie, dose ÷ $AUC_{0\text{-}\infty}$), might well yield CL values that are similar for NL and HL animals, as was previously observed.[17]

The expected decrease in tissue CyA was not uniformly observed. Compared with NL, HL was associated with a decrease in the concentrations of CyA measured in the spleen and heart. Somewhat unexpectedly, however, we observed increases of the drug in the liver and in the kidney of the HL rats. Because the main organ involved in CyA metabolism and elimination is the liver, the current data suggest that hepatic uptake of CyA is facilitated in the presence of HL. This is consistent with the lack of reduced CL in the P407-treated rats, where increased binding to lipoproteins[17] and the expected decrease in hepatic CL were somehow compensated for by another mechanism that increased the hepatic uptake and perhaps the metabolic rate of the drug.

Estimates of postdistributive kp and kb for discernment of the relative CyA uptake of tissues from the blood fluids were calculated (Figure 2). Both parameters suggested that HL restricted the penetration of drug into the spleen and heart, as might be expected based on the decrease in the unbound fraction in plasma previously observed in P407-treated animals.[17] There was some discrepancy between kp and kb for liver and kidney, however (Figure 2). Because CyA binds to blood cells, kb may be more relevant in examining this relationship. The kb indicated a greater relative uptake of drug into the liver in HL animals, whereas for kidney no significant difference was noted. Neither of these observations is in line with the decrease in unbound fraction expected based on the increased binding of CyA to plasma proteins.[17]

The effect of altered lipoprotein levels on the pharmacokinetics of CyA is unclear. Some studies have reported decreases in CL and Vd,[30-33] although in some cases nonspecific radioimmunoassay was used to measure CyA concentrations. It has been shown that in volunteers the CL and Vd of IV-administered CyA are enhanced by the ingestion of a high-fat meal.[20] It was hypothesized that LDL-receptor-mediated uptake was responsible for the increase in CL. In rats, however, studies using human LDL in conjunction with CyA perfused through isolated rat livers failed to show increases in CyA metabolism.[34] The P407 model of HL causes increases primarily in plasma VLDL, and to a lesser degree plasma LDL. It is possible that the cause of the increase in CL postprandially in humans is due not to the association with LDL but rather to higher-triglyceride-containing lipoproteins, such as VLDL and/or chylomicrons.

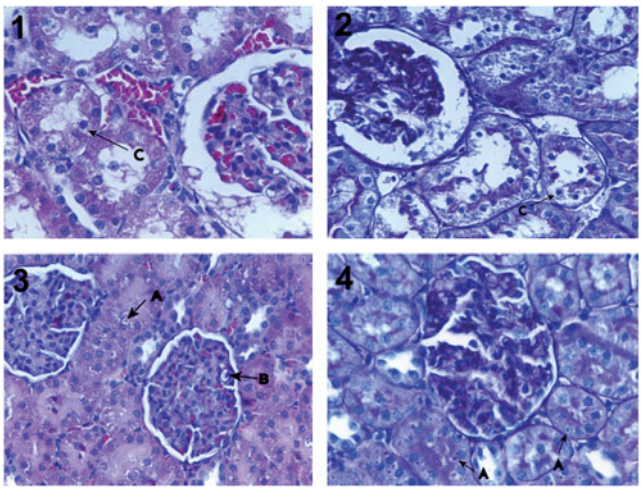

Figure 3. Kidney sections of rats given CyA 20 mg/kg/d for 7 days. Optical magnification is 400×. Panel 1: Section from a normolipidemic rat, stained with H&E. The fine perinuclear vacuolation of tubular epithelial cells, which was present throughout the cortex in animals of this group, is seen (label C). Panel 2: Slice from the same rat as in Panel 1, stained with PAS. The tubular epithelium shows occasional PAS-positive intracytoplasmic droplets (C), although most of the vacuoles are PAS-negative. Panel 3: Section from a hyperlipoproteinemic animal, stained with H&E. Tubular epithelial cells have perinuclear vacuoles with a spherical intracytoplasmic droplet (Label A). Such tubular cell changes appear to be localized to various areas of the cortex, with intervening regions having normal tubular epithelial cell morphology. Label B points to a glomerulus that contains a cell with swollen, finely vacuolated cytoplasm. This is a P407-CyA-related change. Panel 4: Section from the same hyperlipoproteinemic kidney as in Panel 3, stained with PAS. Label A points to the complementary spherical droplets inside vacuolated tubular cell cytoplasm, as seen in Panel 3. These droplets were visibly more prevalent in hyperlipoproteinemic animals than in normolipidemic rats. CyA indicates cyclosporine A; H&E, hematoxylin-eosin; PAS, periodic acid–Schiff; P407, poloxamer 407.

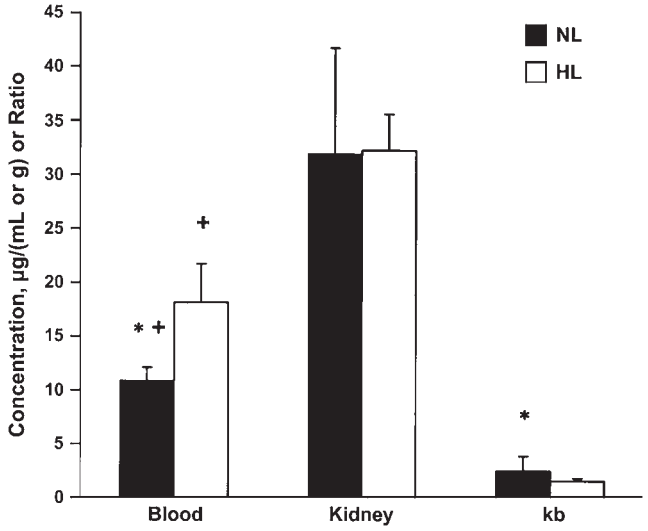

Figure 4. Cyclosporine A concentrations (mean ± SD) in blood and kidney, and kb of NL and HL animals at the end of the treatment period (Day 8) of the repeat dose (20 mg CyA iv/kg/d × 7 days) experiment. * indicates significant difference ($P < .05$) from HL animals in the same parameter; +, significant difference between blood and kidney concentrations in the same animals (HL or NL). NL indicates normolipidemic; HL, hyperlipoproteinemic; kb, tissue-to-blood concentration ratio.

Patients with high total plasma cholesterol levels reportedly have an increased CyA association with plasma LDL and increased CyA-induced renal toxicity compared with NL controls.[32] On the other hand, an in vitro study has suggested that increased VLDL and HDL concentrations could have protective properties against nephrotoxicity and reduce cellular uptake of drug within LLC-PK1 pig kidney cells.[35] The finding of increased CyA uptake in kidney after a single IV dose in HL animals (Table 1, Figure 1), which is in line with previous findings,[32] warranted further study. To explore the significance of the increase in CyA kidney concentrations, the nephrotoxicity experiment was devised and undertaken.

The lack of difference in functional indexes of renal function (Table 2) was consistent with the similar concentrations of CyA in the kidney tissue of NL and HL animals (Figure 4). However, microscopic examination of the stained kidney slices suggested that there were tubular and glomerular changes in the HL animals that were qualitatively not present or not as prevalent in the NL animals (Figure 3). There were also some nonspecific indications from the body weight changes, in which HL rats had decreases in body weight compared with other groups, and predose measurements, which suggested that CyA was more toxic when administered to HL rats (Figure 4). Furthermore, the body weights of these animals at day 7 were significantly lower than those of any of the other groups of animals (Figure 5). HL by itself was not responsible for the decline in body weight, because the body weights of the saline-treated HL animals were not significantly different from those of the saline-treated NL animals. Because at low filtration rates the tubular secretion of creatinine can become significant, the functional severity of the CyA-related nephrotoxicity in HL rats may have been partially obscured.[36]

The results obtained with repeated doses matched well with findings of other investigators. The adverse effects on kidney function in a genetically fixed rat model given a high cumulative dose of CyA (10 mg/kg/d orally for 21 days) were more profound in HL rats than in NL rats.[37] Plasma creatinine and urea concentrations were higher in the HL group, body weights decreased with repeated doses, and there was more evidence of microscopic damage in the HL kidneys. The CyA AUC on day 21 was not significantly different for NL and HL rats.[37] The study differed from ours in that tissue concentrations were not measured. Kidney CyA concentrations have been reported in NL rat kidney, where a mean of 13.5 µg CyA/g was present after 7 days of treatment with 20 mg/kg/

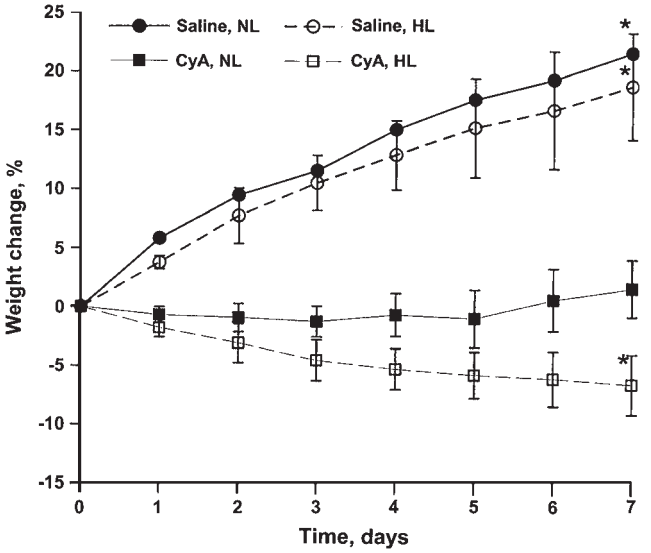

Figure 5. Changes in body weight gain of NL or HL rats studied in the nephrotoxicity experiment, expressed as a percentage of body weight measured on day 0, in rats treated with 20 mg/kg/d CyA or saline. * indicates significant difference between the body weights on day 7 and day 0 ($P < .05$). NL indicates normolipidemic; HL, hyperlipoproteinemic; CyA, cyclosporine A.

day orally once a day.[38] In contrast, in the current study 32.0 ± 9.86 μg/g was reported in the kidney (Figure 4). Considering 29% as the mean oral bioavailability of CyA in rats,[17] the previous reported value in the kidney[38] is consistent with that observed here. After 14 days of treatment, kidney concentrations were 36.6 μg/mL,[38] which was very similar to that observed in the present study (Figure 4). Functionally, this was accompanied by a mean CLcr of 3.45 mL/min/kg,[38] close to that (5.4 mL/min/kg) noted here (Table 2).

The kb of CyA in the kidney in HL and NL rats after 7 days of treatment with 20 mg/kg/d (Figure 4), similar to the kb of AUC and postdistributive concentrations after a single 5 mg/kg dose (Figure 1, Table 1), was considerably greater than 1. After a single dose of drug, the postdistributive kb in kidney (Figure 2) was significantly higher in HL animals, but not NL animals, given repeated doses of CyA (Figure 4). This suggests saturation of the binding of CyA to the kidney tissue in the HL animals. Indeed, saturation of CyA binding to tissues, including kidney, has been reported previously.[39] The cause of the reduced body weight of the HL rats cannot be determined from this study. It is of note that the body weight changes seemed to diverge for the NL and HL rats given CyA from day 1 onward (Figure 5) and that after a single dose of CyA more drug was in the kidney of HL animals (Table 1).

Conclusion

In conclusion, single IV doses of CyA to HL animals were associated with increased blood, plasma, kidney, and liver concentrations, and reduced spleen and heart concentrations, compared with NL animals. After 7 days of IV administration of 20 mg CyA/kg/g, HL and NL rats experienced equivalent decreases in urea and creatinine CLs. However, HL animals given repeated doses of CyA experienced a reduction in body weight that was significant compared with that of NL animals and that was not caused by HL itself. Further studies into the cause of the increased liver concentrations in the face of HL, despite a decrease in the plasma unbound fraction, are warranted and could help to explain the increased CL of CyA in humans in the postprandial state.

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