Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine

Background: **Cyclosporine (INN, ciclosporin) increases the systemic exposure of all statins. Therefore rosuvastatin pharmacokinetic parameters were assessed in an open-label trial involving stable heart transplant recipients (≥6 months after transplant) on an antirejection regimen including cyclosporine. Rosuvastatin has been shown to be a substrate for the human liver transporter organic anion transporting polypeptide C (OATP-C). Inhibition of this transporter could increase plasma concentrations of rosuvastatin. Therefore the effect of cyclosporine on rosuvastatin uptake by cells expressing OATP-C was also examined.**

Methods: **Ten subjects were assessed while taking 10 mg rosuvastatin for 10 days; 5 of these were then assessed while taking 20 mg rosuvastatin for 10 days. Rosuvastatin steady-state area under the plasma concentration–time curve from time 0 to 24 hours [AUC(0-24)] and maximum observed plasma concentration (C_{max}) were compared with values in controls (historical data from 21 healthy volunteers taking 10 mg rosuvastatin). Rosuvastatin uptake by OATP-C–transfected *Xenopus* oocytes was also studied by use of radiolabeled rosuvastatin with and without cyclosporine.**

Results: **In transplant recipients taking 10 mg rosuvastatin, geometric mean values and percent coefficient of variation for steady-state AUC(0-24) and C_{max} were 284 ng · h/mL (31.3%) and 48.7 ng/mL (47.2%), respectively. In controls, these values were 40.1 ng · h/mL (39.4%) and 4.58 ng/mL (46.9%), respectively. Compared with control values, AUC(0-24) and C_{max} were increased 7.1-fold and 10.6-fold, respectively, in transplant recipients. In transplant recipients taking 20 mg rosuvastatin, these parameters increased less than dose-proportionally. Rosuvastatin had no effect on cyclosporine blood concentrations. The in vitro results demonstrate that rosuvastatin is a good substrate for OATP-C–mediated hepatic uptake (association constant, 8.5 ± 1.1 μmol/L) and that cyclosporine is an effective inhibitor of this process (50% inhibition constant, 2.2 ± 0.4 μmol/L when the rosuvastatin concentration was 5 μmol/L).**

Conclusions: **Rosuvastatin exposure was significantly increased in transplant recipients on an antirejection regimen including cyclosporine. Cyclosporine inhibition of OATP-C–mediated rosuvastatin hepatic uptake may be the mechanism of the drug-drug interaction. Coadministration of rosuvastatin with cyclosporine needs to be undertaken with caution. (Clin Pharmacol Ther 2004;76:167-77.)**

Steven G. Simonson, MD, Ali Raza, MD, Paul D. Martin, PhD, Patrick D. Mitchell, MA, John A. Jarcho, MD, Colin D. A. Brown, PhD, Amy S. Windass, BSc, and Dennis W. Schneck, MD, PhD *Wilmington, Del, Cheshire and Northumberland, United Kingdom, and Boston, Mass*

From AstraZeneca, Wilmington; AstraZeneca, Alderley Park, Cheshire; Brigham and Women's Hospital, Boston; and University of Newcastle upon Tyne, Northumberland.

Received for publication Dec 4, 2003; accepted March 31, 2004.

Reprint requests: Steven G. Simonson, MD, AstraZeneca R&D Wilmington, 1800 Concord Pike, Wilmington, DE 19803.
E-mail: *steven.simonson@astrazeneca.com*

0009-9236/$30.00

doi:10.1016/j.clpt.2004.03.010

Cyclosporine (INN, ciclosporin) is used in the prophylaxis of organ rejection in allogenic transplants. Statins—inhibitors of 3-hydroxy-3-methylglutaryl–coenzyme A reductase—are frequently coadministered to patients in whom hypercholesterolemia develops after organ transplantation. However, the use of statins in transplant recipients taking cyclosporine is influenced by the potential for pharmacokinetic drug-drug interactions. Cyclosporine is known to increase the systemic

exposure of all statins, which thus increases the risk for myopathy.[1-7]

In humans, organic anion transporting polypeptide C (OATP-C) (also known as OATP2 or LST1 [SLC21A6]) is selectively expressed in the basolateral membrane of the liver[8] and is involved in the hepatic uptake of statins, including pravastatin,[9,10] cerivastatin,[11] and rosuvastatin.[12] Atorvastatin, simvastatin, and lovastatin are effective inhibitors of pravastatin[9] and rosuvastatin[12] uptake by OATP-C; they are also likely to be substrates for this transporter. Cyclosporine has been shown to inhibit cerivastatin uptake by human hepatocytes in culture.[11] Thus cyclosporine inhibition of OATP-C–mediated statin hepatic uptake may explain, at least in part, the drug-drug interactions reported between cyclosporine and statins, although there is also an established cytochrome P450 (CYP) 3A4 interaction for many statins.

Rosuvastatin (Crestor; licensed by AstraZeneca from Shionogi & Co Ltd, Osaka, Japan) is a statin that has been developed for the treatment of patients with dyslipidemia. The efficacy and safety profiles of rosuvastatin have been reported.[13-16] Metabolic transformation plays a minor role in rosuvastatin clearance (CYP2C9 is the principal CYP isozyme involved in the limited metabolism of rosuvastatin), and thus the potential for clinically relevant metabolically mediated drug-drug interactions is low.[17,18] Ninety percent of an orally administered dose of rosuvastatin is recovered as unchanged drug primarily in the feces.[17] The absolute oral bioavailability of rosuvastatin is 20.1%, the estimated hepatic extraction ratio is 0.63, and the volume of distribution at steady state is 134 L.[19]

In this trial, rosuvastatin pharmacokinetic parameters were assessed in stable heart transplant recipients on an antirejection regimen including cyclosporine. Previous studies have demonstrated that *Xenopus* oocytes can be used to study statin uptake after transfection with OATP-C.[12] These cells can be used as a model to investigate potential interaction mechanisms between cyclosporine and rosuvastatin involving this transporter. Therefore the effect of cyclosporine on the uptake of rosuvastatin by OATP-C–transfected *Xenopus* oocytes was also studied.

METHODS

Human trial

Subjects. Subjects were heart transplant recipients aged greater than 18 years. For inclusion into the trial, at least 6 months must have elapsed since the heart transplant and subjects must have been maintained on a stable antirejection regimen including cyclosporine, prednisone (5-10 mg), and azathioprine (50-200 mg), with a total white blood cell count greater than $4.0 \times 10^3/\mu L$ at screening. Cyclosporine doses were titrated to maintain a whole-blood concentration of 150 to 200 ng/mL. The dose range was from 75 mg twice daily to 200 mg twice daily. The dose of cyclosporine (Neoral or Sandimmun; Novartis AG, Basel, Switzerland) must not have varied in excess of 30% from the lowest daily dosing regimen during the 2 months before screening. Prednisone and azathioprine were administered daily, and the doses remained constant over the trial period. Subjects with major posttransplant complications or unstable medical conditions were excluded. After completion of rosuvastatin treatments, the subjects resumed their pretrial statin therapies.

Trial design. The trial (AstraZeneca Trial No. 4522IL/0021) used an open-label nonrandomized design. Data from healthy volunteers who were administered 10-mg doses of rosuvastatin to steady state in a previous trial were used as a historical control group.[20] Historical controls were used because transplant recipients could not stop receiving their cyclosporine-containing antirejection regimens. The rosuvastatin assay was the same in both trials.

The trial was designed and monitored in accordance with Good Clinical Practice and the Declaration of Helsinki. An investigational review board (Human Research Committee, Brigham and Women's Hospital, Boston, Mass) approved the protocol before the trial started, and all subjects gave written informed consent. The trial was conducted at a single center (Brigham and Women's Hospital).

Eligibility for the trial was determined at a screening visit. After screening, there was a washout (1-week minimum) of any pretrial statin therapy before the start of the trial. The short washout period was selected to minimize the time off statin therapy. Subjects were given a single oral dose of 10 mg rosuvastatin, followed by 10 once-daily oral doses of 10 mg rosuvastatin (cohort 1); there was an interval of 72 hours between the single- and daily-dosing periods. Cohort 2 was given a single dose and then 10 daily oral doses of 20 mg rosuvastatin, with an interval of 14 days between the single- and daily-dosing periods. The interval allowed pharmacokinetic assessment after the single dose. Subjects in cohort 1 were allowed to participate in cohort 2 provided that the area under the plasma concentration–time curve from time 0 to infinity (AUC) was 750 ng · h/mL or lower and the maximum observed plasma concentration (C_{max}) was 250 ng/mL or lower after the single 20-mg dose.

The rosuvastatin pharmacokinetic profile was determined on the first and last days of rosuvastatin administration. On these days, rosuvastatin was given at 8 AM, after a 12-hour fasting period in the clinical research center. On other days, rosuvastatin was administered on an outpatient basis 1 hour before or 2 hours after breakfast. Throughout the trial, all subjects continued to receive their pretrial cyclosporine regimens every 12 hours, along with their prednisone and azathioprine treatments. Cyclosporine was administered concomitantly with rosuvastatin on the days of blood sampling for pharmacokinetic measurements.

During the trial, subjects were not allowed to take any other concomitant medications unless approved by the investigator (such medications included angiotensin-converting enzyme inhibitors, β-blockers, furosemide, sulfonylureas, anxiolytics, and acid-suppressive drugs; the potential for these drugs to interact with rosuvastatin was considered minimal). Subjects were also required to refrain from any activity that could have predisposed them to spurious elevations in creatine kinase levels (eg, vigorous exercise). In addition, major changes in dietary habits were not permitted, and subjects were to refrain from consuming alcohol and certain foods (eg, grapefruit-containing products, smoked meats, cabbage).

The primary objective of the trial was to determine the pharmacokinetics of single and multiple doses of rosuvastatin in heart transplant recipients on an immunosuppressive regimen including cyclosporine. Secondary objectives were to assess the effect of rosuvastatin on the pharmacokinetics of cyclosporine, to evaluate pharmacodynamics (plasma lipid levels), and to assess the tolerability of this drug combination.

Blood sampling for pharmacokinetic evaluation. Venous blood samples (5 mL) for rosuvastatin assay were taken before and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 48, and 72 hours after administration of the single dose of rosuvastatin and the last daily dose of rosuvastatin. Samples were collected into tubes containing lithium-heparin anticoagulant and centrifuged within 30 minutes; plasma was then harvested from the samples. Plasma samples were mixed 1:1 with 0.1-mol/L sodium acetate buffer (pH 4.0) and stored at −70°C until assay.

Venous blood samples (5 mL) for cyclosporine assay were taken before and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours after administration of cyclosporine on the day before administration of the single dose of rosuvastatin, on the day of administration of the single dose of rosuvastatin, and on the day of administration of the last daily dose of rosuvastatin. Samples were collected into tubes containing ethylenediaminetetraacetic acid and stored at −20°C until assay.

Drug assays. Plasma samples were analyzed for rosuvastatin at Quintiles Ltd (Edinburgh, Scotland, United Kingdom) by a validated method (HPLC with mass spectrometric detection), which has been described elsewhere.[21] The effective limit of quantitation for rosuvastatin was 0.2 ng/mL. The accuracy and precision of the analytic methods were ensured on the basis of the results for spiked quality-control samples. At all concentrations assessed (0.3, 1.0, 15.0, 25.0, and 250.0 ng/mL), the mean inaccuracy values were less than 3.7% and the mean imprecision values were less than 6.9%.

Whole-blood samples were analyzed for cyclosporine at Phoenix International Life Sciences Inc (Montreal, Quebec, Canada) by a validated method (HPLC with mass spectrometric detection). The limit of quantitation for cyclosporine was 10 ng/mL. On the basis of the results for spiked quality-control samples, the mean inaccuracy values were less than 7% and the mean imprecision values were less than 9.2% at all concentrations assessed (30.0, 400.4, and 800.8 ng/mL).

Pharmacokinetic parameters. Rosuvastatin pharmacokinetic parameters measured included AUC and area under the plasma concentration–time curve from time 0 to 24 hours [AUC(0-24)], C_{max}, time to C_{max} (t_{max}), and terminal elimination half-life ($t_{½}$). Cyclosporine pharmacokinetic parameters measured included area under the whole-blood concentration–time curve from time 0 to 12 hours [AUC(0-12)], C_{max}, and $t_{½}$.

AUC(0-24) and AUC(0-12) were determined by use of the linear trapezoidal rule. AUC was determined by use of the linear trapezoidal rule up to the last measurable concentration and thereafter by extrapolation of the terminal elimination phase to infinity. C_{max} and t_{max} were determined by visual inspection of the concentration-time curves, and $t_{½}$ was calculated as $0.693/\lambda_z$ (in which λ_z is the terminal elimination rate constant derived from log-linear regression of the terminal portion of the concentration-time curve where there were sufficient data).

Statistical methods. Summary statistics are reported for the pharmacokinetic parameters. The main comparison was between rosuvastatin AUC(0-24) and C_{max} after multiple dosing in this trial and the same parameters measured in healthy volunteers after 14 once-daily doses of 10 mg rosuvastatin in a previous trial.[20]

Conclusions regarding rosuvastatin dosing affecting cyclosporine blood concentrations are based on the 90% confidence intervals (CIs) for the postrosuvastatin/

prerosuvastatin ratios for cyclosporine AUC(0-12) and C_{max}.

Pharmacodynamic evaluation. Venous blood samples were also collected for analysis of plasma lipid levels before and after the rosuvastatin dosing periods. Subjects fasted for at least 12 hours before samples were taken. Plasma samples were analyzed for low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations. Samples were analyzed at the Brigham and Women's Hospital clinical laboratory. This laboratory was certified for standardization of lipid analysis as specified by the Standardization Program of the Centers for Disease Control and Prevention (Atlanta, Ga) and the National Heart, Lung, and Blood Institute (Bethesda, Md). TC, HDL-C, and TG concentrations were measured as specified by the Standardization Program.[22] LDL-C values were estimated by use of the Friedewald equation.

Tolerability. The following assessments were performed or obtained: adverse event questioning and subject reports, medical examinations, clinical laboratory data, and electrocardiograms.

In vitro study

Materials. Tritium-labeled rosuvastatin (specific activity, 2.8 TBq/mmol) was supplied by AstraZeneca (Macclesfield, United Kingdom). Collagenase (type A) was supplied by Roche Molecular Biochemicals (Mannheim, Germany). All other chemicals were supplied by Sigma Chemical Company (St Louis, Mo).

Xenopus oocytes. Stage V to VI morphologically healthy oocytes from *Xenopus laevis* were obtained from the South African Xenopus Facility (Knysna, Republic of South Africa).

Study design. Uptake of tritium-labeled rosuvastatin into oocytes expressing OATP-C and into water-injected control oocytes was measured over a range of rosuvastatin concentrations (0-100 μmol/L). Uptake of [^{3}H]rosuvastatin (5 μmol/L) into oocytes expressing OATP-C was also measured in the presence of a range of cyclosporine concentrations (0-50 μmol/L).

Synthesis of complementary ribonucleic acid. Human *OATP-C*1a* complementary deoxyribonucleic acid was used as a template for complementary ribonucleic acid (cRNA) synthesis. Plasmids were linearized with a single restriction digest upstream of the T7 promoter. In vitro transcription of the linear complementary deoxyribonucleic acid template was achieved with the mMessage mMachine T7 Kit (Ambion, Huntingdon, United Kingdom). cRNA was diluted to a final concentration of 0.4 μg/μL with sterile ribonuclease-free water before storage at −80°C.

Preparation of oocytes. Oocytes were treated with collagenase for 1 to 2 hours at 20°C until the follicular layer had been removed. The progress of the digestion was monitored at regular intervals by microscopic examination. After overnight storage at 18°C in Barth's solution [88-mmol/L sodium chloride, 1-mmol/L potassium chloride, 0.82-mmol/L magnesium sulfate, 2.4-mmol/L sodium bicarbonate, 0.41-mmol/L calcium chloride, 0.33-mmol/L calcium nitrate, and 10-mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)–tris(hydroxymethyl)aminomethane, pH 7.6], oocytes were injected with either 50 nL of 0.4-ng/nL cRNA (20 ng cRNA) or 50 nL of water (control). Oocytes were then stored at 18°C in Barth's solution supplemented with gentamicin (20 μg/mL). They were allowed 2 to 3 days to translate the cRNA and express the protein at the plasma membrane.

Assessment of rosuvastatin uptake by oocytes. Ten oocytes per experimental condition were placed in a 5-mL test tube with a small volume of Barth's solution. The Barth's solution was removed and replaced with 300 μL of Barth's solution containing either rosuvastatin (0-100 μmol/L) or rosuvastatin (5 μmol/L) plus cyclosporine (0-50 μmol/L). Each uptake solution contained [^{3}H]rosuvastatin at 3 μCi/mL. The oocytes were incubated at 18°C for 1 hour. Initial experiments had established that the uptake of radioactivity was linear over a 240-minute period of incubation. After the incubation period, the uptake solution was aspirated and the oocytes were washed 3 times by the addition of 2.5 mL of ice-cold control solution to remove the remaining labeled substrate and to prevent further uptake. Individual oocytes were placed into vials containing 500 μL of 2% sodium dodecyl sulfate and allowed to lyse. This was followed by the addition of 5 mL of scintillation cocktail. The ^{3}H content was then measured by scintillation spectrophotometry.

To measure specific OATP-C–mediated uptake of rosuvastatin, the uptake of rosuvastatin was measured in parallel in 10 oocytes injected with cRNA and 10 oocytes injected with water for each substrate concentration. The uptake into water-injected oocytes was then subtracted from the uptake into cRNA-injected oocytes.

Statistical methods. Results are expressed as mean ± SEM. The model used to relate the rate of uptake to rosuvastatin concentration in the media was as follows: $V_0 = (V_{max} \cdot S)/(Ka + S)$, where V_0 is the rate of uptake (in picomoles per oocyte per hour), V_{max} is the maximum rate of uptake (in picomoles per oocyte per

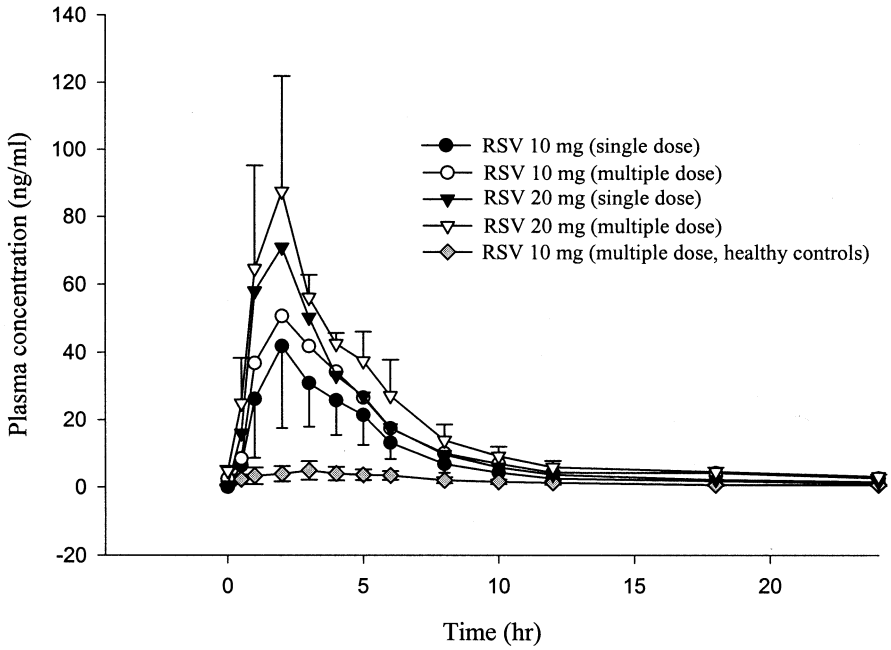

Fig 1. Rosuvastatin (RSV) plasma concentration–time profiles after single and multiple dosing in heart transplant recipients (cohort 1, n = 10; cohort 2, n = 5) and after multiple dosing in healthy volunteers (n = 21). Concentrations are given as geometric means on a linear scale; *error bars* are based on SDs of log-transformed data.

hour), K_a is the association constant, and S is the rosuvastatin concentration in the media (in micromoles per liter). Curve fitting and determination of K_a and 50% inhibition constant (IC_{50}) values were achieved by nonlinear regression analysis (Levenberg-Marquardt). All curve-fitting and rate constant determinations were performed after subtraction of rosuvastatin uptake into water-injected oocytes from the total uptake measured in cRNA-injected oocytes.

RESULTS

Human trial

Demographics. Ten subjects entered cohort 1 and completed this phase of the trial. There were 9 men and 1 woman. Their mean age, height, and weight were 53.2 years (range, 30-69 years), 169.5 cm (range, 156.0-182.8 cm), and 89.0 kg (range, 68.1-109.5 kg), respectively. Nine of the subjects were white and 1 was Hispanic.

Six subjects entered cohort 2 (5 of these had previously participated in cohort 1) and 5 completed this phase of the trial (1 subject who had not participated in cohort 1 was withdrawn because of protocol noncompliance).

Subjects in both cohorts were receiving a variety of other medications in addition to immunosuppressive treatment. The other drug categories commonly prescribed included antihypertensives (including diltiazem), oral hypoglycemics, and inhibitors of gastric acid secretion. Half of the subjects in cohort 1 were coadministered diltiazem.

Pharmacokinetic parameters: Rosuvastatin. Mean plasma concentrations of rosuvastatin over time are depicted in Fig 1. Summary pharmacokinetic parameters of rosuvastatin are presented in Table I.

Compared with healthy controls, geometric mean (gmean) steady-state AUC(0-24) and C_{max} values were increased 7.1- and 10.6-fold, respectively, in transplant recipients taking 10 mg rosuvastatin (Table I). Individual AUC(0-24) values ranged from 175 to 431 ng · h/mL (2.5-fold range); values for C_{max} ranged from 25 to 104 ng/mL (4-fold range). Rosuvastatin $t_{½}$ was not prolonged in transplant recipients compared with controls (Table I).

Geometric mean AUC(0-24) and C_{max} values were increased in transplant recipients taking 20 mg rosuvastatin compared with the values for those taking 10 mg

Table I. Summary pharmacokinetic parameters of rosuvastatin in heart transplant recipients on an antirejection regimen including cyclosporine and in healthy controls not taking cyclosporine

Rosuvastatin parameter	*Summary statistic*	*Healthy controls**	*Heart transplant recipients*	
		RSV 10 mg	*RSV 10 mg*	*RSV 20 mg*
Single-dose rosuvastatin		n = 21	n = 10	n = 6
AUC(0-24) (ng · h/mL)	Geometric mean and %CV	NA	197 (38.5)	308 (34.0)
C_{max} (ng/mL)	Geometric mean and %CV	NA	39.8 (53.9)	66.5 (49.4)
AUC (ng · h/mL)	Geometric mean and %CV	NA	267 (19.2)†	375 (34.0)‡
t_{max} (h)	Median and range	NA	2.00 (1.00-4.00)	2.00 (1.00-2.00)
$t_{1/2}$ (h)	Arithmetic mean and SD	NA	17.1 (6.14)†	19.4 (3.95)‡
Multiple-dose rosuvastatin		n = 21	n = 10	n = 5
AUC(0-24) (ng · h/mL)	Geometric mean and %CV	40.1 (39.4)	284 (31.3)	424 (21.7)
C_{max} (ng/mL)	Geometric mean and %CV	4.58 (46.9)	48.7 (47.2)	83.4 (37.3)
AUC (ng · h/mL)	Geometric mean and %CV	71.8 (30.9)§	361 (16.9)‖	463 (4.08)‡
t_{max} (h)	Median and range	3.00 (1.00-6.00)	2.00 (1.00-4.00)	2.00 (1.00-2.00)
$t_{1/2}$ (h)	Arithmetic mean and SD	31.3 (12.0)§	14.8 (4.05)‖	20.2 (5.37)‡

RSV, Rosuvastatin; AUC(0-24), area under plasma concentration–time curve from time 0 to 24 hours; C_{max}, maximum observed plasma concentration; AUC, area under plasma concentration–time curve from time 0 to infinity; t_{max}, time to maximum observed plasma concentration; $t_{1/2}$, terminal elimination half-life; %CV, coefficient of variation expressed as percentage of geometric mean; NA, not available.

*Results taken from a previous rosuvastatin trial.[20]

†n = 4 (values for some subjects could not be calculated because no reliable estimate of terminal elimination could be obtained as a result of concentrations below the sensitivity of the assay).

‡n = 3 (values for some subjects could not be calculated because no reliable estimate of terminal elimination could be obtained as a result of concentrations below the sensitivity of the assay).

§n = 16 (values for some subjects could not be calculated because no reliable estimate of terminal elimination could be obtained as a result of concentrations below the sensitivity of the assay).

‖n = 5 (values for some subjects could not be calculated because no reliable estimate of terminal elimination could be obtained as a result of concentrations below the sensitivity of the assay).

Table II. Postrosuvastatin prerosuvastatin ratios and 90% confidence intervals for cyclosporine AUC(0-12) and C_{max} in heart transplant recipients

Cyclosporine parameter	*Heart transplant recipients*	
	*RSV 10 mg (n = 6)**	*RSV 20 mg*
Single-dose rosuvastatin	n = 6*	n = 6*
AUC(0-12) (ng · h/mL)	1.10 (0.99-1.24)	1.07 (1.00-1.15)
C_{max} (ng/mL)	1.22 (0.87-1.70)	1.06 (0.84-1.33)
Multiple-dose rosuvastatin	n = 6*	n = 5*
AUC(0-12) (ng · h/mL)	1.04 (0.90-1.20)	0.97 (0.81-1.15)
C_{max} (ng/mL)	1.11 (0.78-1.58)	0.88 (0.60-1.27)

AUC(0-12), Area under plasma concentration–time curve from time 0 to 12 hours.

*Initial samples for cyclosporine assessment were collected in error as plasma. After detection of the error, samples of whole blood were collected and analyzed. For this reason, Table II includes only data derived from subjects for whom complete pharmacokinetic parameters from whole blood were available at each visit.

rosuvastatin, but the increase was less than dose-proportional (Table I). After multiple dosing, the gmean values for the AUC(0-24) and C_{max} 20-mg/10-mg ratio (calculated for subjects who took both doses of rosuvastatin) were 1.38 and 1.49, respectively.

The rosuvastatin accumulation ratios [AUC(0-24) after multiple dosing/AUC(0-24) after single dose] were 1.44 (90% CI, 1.25-1.67) and 1.28 (90% CI, 0.97-1.70) after the 10- and 20-mg doses, respectively. The temporal change ratios [AUC(0-24) after multiple dosing/AUC after single dose] were 1.11 (90% CI, 0.89-1.39) and 0.85 (90% CI, 0.49-1.47) after the 10- and 20-mg doses, respectively.

Pharmacokinetic parameters: Cyclosporine. Cyclosporine AUC(0-12) and C_{max} values before and after rosuvastatin administration were similar, as illustrated by postrosuvastatin/prerosuvastatin ratios near unity (Table II).

Table III. Mean and SD of lipid levels and percent change from baseline in heart transplant recipients on an antirejection regimen including cyclosporine

	Heart transplant recipients					
	RSV 10 mg (n = 10)			*RSV 20 mg (n = 5)*		
Lipid parameter	*Baseline*	*Final*	*% Change*	*Baseline*	*Final*	*% Change*
LDL-C						
mmol/L	3.88 (1.05)	2.65 (0.89)	–29.0 (26.2)	4.08 (0.77)	2.63 (0.75)	–34.5 (21.1)
mg/dL	150 (40.7)	103 (34.5)		158 (29.7)	102 (28.8)	
TC						
mmol/L	6.70 (1.54)	5.05 (0.78)	–24.1 (11.5)	6.22 (0.79)	4.83 (0.44)	–21.6 (10.5)
mg/dL	259 (59.4)	195 (30.2)		241 (30.5)	187 (17.2)	
HDL-C						
mmol/L	1.23 (0.47)	1.31 (0.47)	11.3 (9.63)	1.22 (0.43)	1.29 (0.46)	6.4 (10.8)
mg/dL	47.4 (18.2)	50.5 (18.2)		47.0 (16.6)	49.8 (17.8)	
TG						
mmol/L	3.37 (2.17)	2.18 (1.14)	–32.1 (23.0)	2.34 (1.80)	2.17 (1.37)	0.55 (40.5)
mg/dL	298 (192)	193 (101)		207 (160)	192 (121)	

LDL-C, Low-density lipoprotein cholesterol; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

Pharmacodynamic parameters: Lipid levels. The lipid levels and percent change from baseline are summarized in Table III. LDL-C and TC levels were substantially lowered and HDL-C levels were increased with both doses of rosuvastatin. TG levels were substantially lowered with 10 mg rosuvastatin but not with 20 mg rosuvastatin, although the final values were similar after both doses.

Tolerability. In this trial, both doses of rosuvastatin were well tolerated in heart transplant recipients on an antirejection regimen including cyclosporine. One subject had an elevated ALT value at baseline that increased during the 10-mg dosing period. This subject also participated in the 20-mg dosing period, during which the ALT level remained within normal limits. There were no cases of myopathy, and none of the subjects had serum creatine kinase concentrations greater than the normal range after administration of rosuvastatin. No serious adverse events were reported.

In vitro study

The uptake of [^{3}H]rosuvastatin into oocytes expressing OATP-C is shown in Fig 2. The data show that rosuvastatin is a good substrate for OATP-C. At each concentration assessed, the uptake of rosuvastatin into oocytes expressing OATP-C was approximately 10-fold greater than the uptake into water-injected control oocytes. Nonlinear least squares regression analysis of the OATP-C–mediated component of rosuvastatin uptake yielded an apparent K_a of 8.5 ± 1.1 μmol/L.

The effect of cyclosporine on the kinetics of OATP-C–mediated [^{3}H]rosuvastatin (5 μmol/L) uptake is shown in Fig 3. The results demonstrate that cyclosporine is an effective inhibitor of OATP-C–mediated rosuvastatin uptake. The IC_{50} for the inhibitory process was 2.2 ± 0.4 μmol/L.

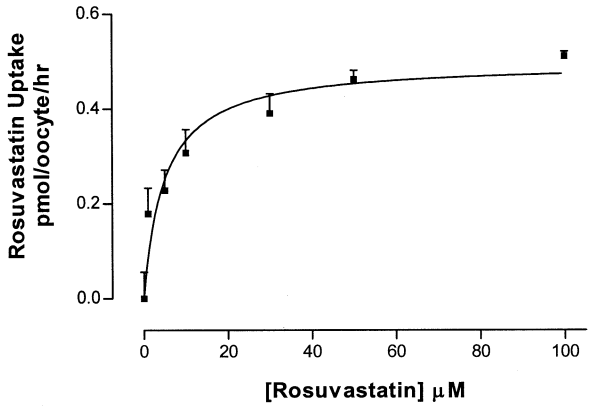

Fig 2. Uptake of tritium-labeled rosuvastatin into oocytes expressing organic anion transporting polypeptide C (OATP-C). Nonlinear least squares regression analysis of the data from 8 independent experiments yielded an apparent association constant (K_a) of 8.5 ± 1.1 μmol/L. (Each data point represents the mean ± SEM of [^{3}H]rosuvastatin uptake into 10 oocytes from a single animal.)

DISCUSSION

This trial showed that, compared with historical controls, rosuvastatin gmean steady-state AUC(0-24) and

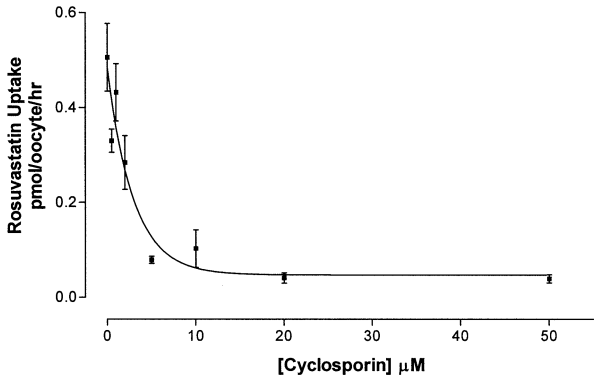

Fig 3. Effect of cyclosporine on kinetics of OATP-C–mediated [^{3}H]rosuvastatin uptake. The inhibition constant (IC_{50}) for the cyclosporine inhibitory process was 2.2 ± 0.4 μmol/L. (Each data point represents the mean ± SEM of 8 to 10 oocytes per condition from a single experiment representative of the data from 3 independent experiments.)

C_{max} values were increased 7.1-fold and 10.6-fold, respectively, in heart transplant recipients taking 10 mg rosuvastatin. The increases in AUC(0-24) and C_{max} in transplant recipients taking 20 mg rosuvastatin (compared with 10 mg rosuvastatin) were less than dose-proportional. The lack of change in the temporal change ratio indicates no time dependence of rosuvastatin pharmacokinetics in the presence of cyclosporine.

The historical control group was considered a good comparator for steady-state exposure. However, the mean t½ estimate of 31 hours in the control group (Table I) was atypical of the t½ estimates observed across a range of phase 1 trials across all doses. A typical t½ estimate was 20 hours.[19] The mean t½ estimate in the present trial was 15 hours at the 10-mg dose and 20 hours at the 20-mg dose (Table I). It is likely that the t½ was unchanged in the presence of cyclosporine. The t½ is dependent on distribution volume and clearance. The t½ would remain constant if decreases in both of these parameters occurred at the same time and to a similar extent. If rosuvastatin t½ was reduced in the presence of cyclosporine, then either a reduction in distribution volume or an increase in clearance must have occurred. An increase in clearance is highly unlikely, and, therefore, a reduction in distribution volume would have had to occur to account for the change. This is much more likely and could be accounted for by inhibition of rosuvastatin hepatic uptake as discussed later. Rosuvastatin uptake clearance in rat hepatocytes is high and carrier-mediated.[23] The liver is likely to be a major contributor to rosuvastatin distribution volume.

The historical controls were healthy young adult male and female volunteers participating in a trial designed to assess the effect of time of day of administration on the pharmacokinetics and pharmacodynamics of rosuvastatin. Age and sex have been shown to have no significant effect on rosuvastatin pharmacokinetics.[24] The differences in age and sex between the historical controls and the transplant recipients in this trial cannot account for the differences in pharmacokinetics between the 2 groups.

The transplant recipients in this trial received a variety of additional drugs such as antihypertensives, oral hypoglycemics, and inhibitors of gastric acid secretion, as well as prednisone and azathioprine. On the basis of the current literature, the comedications used are unlikely to possess any potential for interaction with rosuvastatin. Diltiazem (a CYP3A4 and P-glycoprotein inhibitor) is commonly prescribed to heart transplant recipients taking cyclosporine. To rule out confounding effects of this drug on the outcomes of the current trial, the results in subjects taking diltiazem (n = 5; rosuvastatin AUC, 30.6 ng · h/mL; percent coefficient of variation, 48.8%) were compared with those in subjects who did not receive diltiazem (n = 5; rosuvastatin AUC, 51.7 ng · h/mL; percent coefficient of variation, 45.7%). Rosuvastatin plasma concentrations were substantially increased in both groups but less so in the subjects taking diltiazem.

The mechanism(s) by which cyclosporine might increase the systemic plasma concentrations of rosuvastatin include enhanced absorption from the gastrointestinal tract, reduced hepatic extraction, reduced systemic clearance, or some combination of these effects. The intestinal absorptive process for rosuvastatin has not been well characterized. Studies with Caco-2 cell monolayers have shown net secretion across these cells from the basolateral-to-apical surface (AstraZeneca, data on file).

Cyclosporine is a substrate for and an inhibitor of P-glycoprotein–mediated transport.[25,26] Rosuvastatin-lactone is, but rosuvastatin is not, a substrate for P-glycoprotein transport.[27] In humans, rosuvastatin-lactone circulates at plasma concentrations approximately 10% of those of rosuvastatin.[28] Digoxin is a substrate for P-glycoprotein, but rosuvastatin had no effect on digoxin pharmacokinetics in healthy volunteers.[29] If rosuvastatin-lactone had any relevant inhibitory effect on P-glycoprotein transport, a change in digoxin disposition should have been observed. In addition, ketoconazole (a known inhibitor of P-glycoprotein) had no effect on rosuvastatin pharmacokinetics in healthy volunteers.[30] These results

strongly suggest that cyclosporine inhibition of P-glycoprotein cannot be the mechanism for the interaction observed in this trial. It is possible that cyclosporine inhibits an as yet undefined intestinal transporter for rosuvastatin and that this inhibition enhances the bioavailability of rosuvastatin.

Cyclosporine is also an inhibitor of OATP-C (OATP2),[11] an organic anion transport protein thought to be involved in the hepatic uptake of rosuvastatin.[12] The results of these in vitro experiments demonstrate that rosuvastatin is a good substrate for OATP-C. In addition, the results show that cyclosporine is a potent inhibitor of OATP-C–mediated rosuvastatin uptake in *Xenopus* oocytes. The mean total plasma rosuvastatin C_{max} at steady state in the healthy volunteer trial was about 5 ng/mL after administration of 10-mg doses (Table I). Rosuvastatin plasma protein binding is approximately 90% (AstraZeneca, data on file). The mean systemic free concentration of rosuvastatin at C_{max} is approximately 0.5 ng/mL (1 nmol/L). The systemic free plasma concentration of cyclosporine at C_{max} is approximately 100 nmol/L. Thus the free concentration of cyclosporine is about 100 times that of rosuvastatin. Previous studies examining the inhibitory effect of cyclosporine on cerivastatin uptake by OATP-C–transfected cells have estimated the cyclosporine inhibition constant to range from 280 to 690 nmol/L.[11] Thus the affinity of cyclosporine for OATP-C is higher than that for rosuvastatin. Portal vein concentrations of cyclosporine and rosuvastatin may be much higher than systemic concentrations after oral administration (in the clinical trial, both drugs were taken together by oral administration). The mechanism by which cyclosporine inhibits OATP-C is unknown. It is possible that inhibition may also be dependent on cyclosporine hepatic concentrations, which may be much higher than plasma concentrations. The circulating plasma concentrations of rosuvastatin are well below the K_m value determined in the in vitro studies. This observation indicates that the hepatic uptake of rosuvastatin is dominated by transporter uptake at therapeutic plasma concentrations. The results indicate that the increase in rosuvastatin plasma concentrations in the presence of cyclosporine is, at least in part, mediated by cyclosporine inhibition of hepatic rosuvastatin uptake by OATP-C.

Coadministration of cerivastatin to kidney transplant patients treated with cyclosporine and other immunosuppressive agents resulted in a 3.8-fold increase in AUC and a 5-fold increase in C_{max} compared with values in healthy volunteers.[6]

Shitara et al[11] investigated the mechanism for the drug-drug interaction between cyclosporine and cerivastatin by use of cultured human hepatocytes. Cyclosporine was found to be a potent and effective inhibitor of cerivastatin uptake by these cells and of cerivastatin uptake by Madin-Darby canine kidney II (MDCKII) cells expressing the OATP-C transporter. Cyclosporine inhibition of cerivastatin metabolism was also examined, and cyclosporine inhibitory concentrations were found to be much higher than those inhibiting cerivastatin transport. The investigators concluded that the main mechanism for the interaction was cyclosporine inhibition of cerivastatin hepatic transport.

Yamazaki et al[31] have demonstrated that the canalicular multispecific organic anion transporter (cMOAT) contributes to the biliary secretion of pravastatin. Rosuvastatin may also be a substrate for this transporter, although no studies to date have demonstrated an interaction. Cyclosporine inhibition of this transporter could also contribute to the mechanism of the interaction for pravastatin and possibly for rosuvastatin.

Cyclosporine is an inhibitor of CYP3A4.[1] Atorvastatin, simvastatin, and lovastatin are all substrates for CYP3A4,[1] and cyclosporine inhibition of CYP3A4 may contribute to the interactions reported between cyclosporine and these statins.[2-4] Rosuvastatin is not a substrate for CYP3A4 metabolism.[18] This statement is supported by the lack of any relevant interaction of rosuvastatin with ketoconazole and erythromycin in vivo.[30,32] Thus the increase in rosuvastatin plasma concentrations in the presence of cyclosporine cannot be due to inhibition of CYP3A4.

The exploratory analysis of lipid parameters in this trial indicates a substantial lipid response to rosuvastatin despite the potential inhibition of hepatic uptake by cyclosporine. Nine of the subjects in cohort 1 were taking statins before entering the trial (7 were taking pravastatin and 2 were taking atorvastatin). The washout period was 7 days, and the baseline lipid values after statin discontinuation likely underestimate steady-state values after statin discontinuation. The short washout period was selected to minimize the time off statin therapy. An adequate washout time to establish baseline lipid levels would have required at least 4 weeks off statin therapy. A washout period of this length was not considered appropriate for this at-risk population, and the lipid measurements were a secondary end point of the trial. The measurements were obtained to establish the lipid-lowering activity of rosuvastatin in this setting. Maximal response to statin therapy would require 4 weeks of treatment, because approximately 70% and 90% of the full response are seen at 1 and 2 weeks of treatment, respectively.[14] Thus

the change in LDL-C levels after the 10 days of rosuvastatin treatment in this trial is unlikely to represent the full effect. Despite the pharmacokinetic interaction, the lipid response to rosuvastatin was substantial.

In summary, rosuvastatin systemic exposure was significantly increased in transplant recipients receiving an antirejection regimen including cyclosporine: steady-state AUC(0-24) and C_{max} values were increased 7.1- and 10.6-fold, respectively, compared with values in healthy volunteers taking only 10 mg rosuvastatin. Cyclosporine inhibition of rosuvastatin hepatic uptake by OATP-C may be responsible, in part, for the mechanism of the interaction. Coadministration of rosuvastatin with cyclosporine needs to be undertaken with caution.

We thank Quintiles Ltd (Edinburgh, Scotland, United Kingdom) for performing the rosuvastatin assays, Phoenix International Life Sciences Inc (Montreal, Quebec, Canada) for performing the cyclosporine assays, Brigham and Women's Hospital (Boston, Mass) for performing the lipid assays, and Elizabeth Eaton, PhD, for assistance with manuscript preparation.

Steven G. Simonson, Ali Raza, Paul D. Martin, Patrick D. Mitchell, and Dennis W. Schneck are employees of AstraZeneca and hold stock in the company. John A. Jarcho, Colin D. A. Brown, and Amy S. Windass received research support from AstraZeneca.

References

1. Martin J, Krum H. Cytochrome P450 drug interactions within the HMG-CoA reductase inhibitor class. Are they relevant? Drug Saf 2003;26:13-21.
2. Åsberg A, Hartmann A, Fjeldså E, Bergan S, Holdaas H. Bilateral pharmacokinetic interaction between cyclosporine A and atorvastatin in renal transplant recipients. Am J Transplant 2001;1:382-6.
3. Arnadottir M, Eriksson LO, Thysell H, Karkas JD. Plasma concentration profiles of simvastatin 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitory activity in kidney transplant recipients with and without ciclosporin. Nephron 1993;65:410-3.
4. Olbricht C, Wanner C, Eisenhauer T, Kliem V, Doll R, Boddaert M, et al. Accumulation of lovastatin, but not pravastatin, in the blood of cyclosporine-treated kidney graft patients after multiple doses. Clin Pharmacol Ther 1997;62:311-21.
5. Regazzi MB, Iacona I, Campana C, Raddato V, Lesi C, Perani G, et al. Altered disposition of pravastatin following concomitant drug therapy with cyclosporin A in transplant recipients. Transplant Proc 1993;25:2732-4.
6. Mück W, Mai I, Fritsche L, Ochmann K, Rohde G, Unger S, et al. Increase in cerivastatin systemic exposure after single and multiple dosing in cyclosporine-treated kidney transplant recipients. Clin Pharmacol Ther 1999; 65:251-61.
7. Park JW, Siekmeier R, Lattke P, Merz M, Mix C, Schuler S, et al. Pharmacokinetics and pharmacodynamics of fluvastatin in heart transplant recipients taking cyclosporine A. J Cardiovasc Pharmacol Ther 2001;6:351-61.
8. Konig J, Cui Y, Nies AT, Keppler D. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. Am J Physiol Gastrointest Liver Physiol 2000;278:G156-64.
9. Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP, et al. A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. J Biol Chem 1999;274:37161-8.
10. Nakai D, Nakagomi R, Furuta Y, Tokui T, Abe T, Ikeda T, et al. Human liver-specific organic anion transporter, LST-1, mediates uptake of pravastatin by human hepatocytes. J Pharmacol Exp Ther 2001;297:861-7.
11. Shitara Y, Itoh T, Sato H, Li AP, Sugiyama Y. Inhibition of transporter-mediated hepatic uptake as a mechanism for drug-drug interaction between cerivastatin and cyclosporin A. J Pharmacol Exp Ther 2003;304:610-6.
12. Brown CDA, Windass A, Bleasby K, Lauffart B. Rosuvastatin is a high affinity substrate of hepatic organic anion transporter OATP-C [abstract]. Atheroscler Suppl 2001;2:90.
13. Brewer HB Jr. Benefit-risk assessment of rosuvastatin 10 to 40 milligrams. Am J Cardiol 2003;92:23K-29K.
14. Schneck DW, Knopp RH, Ballantyne CM, McPherson R, Chitra RR, Simonson SG. Comparative effects of rosuvastatin and atorvastatin across their dose ranges in patients with hypercholesterolemia and without active arterial disease. Am J Cardiol 2003;91:33-41.
15. Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E, et al. Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR Trial). Am J Cardiol 2003;93:152-60.
16. McKenney JM, Jones PH, Adamczyk MA, Cain VA, Bryzinski BS, Blasetto JW, et al. Comparison of the efficacy of rosuvastatin versus atorvastatin, simvastatin, and pravastatin in achieving lipid goals: results from the STELLAR trial. Curr Med Res 2003;19:689-98.
17. Martin PD, Warwick MJ, Dane AL, Hill SJ, Giles PB, Phillips PJ, et al. Metabolism, excretion, and pharmacokinetics of rosuvastatin in healthy adult male volunteers. Clin Ther 2003;25:2822-35.
18. McCormick AD, McKillop D, Butters CJ, Miles GS, Baba T, Touchi A, et al. ZD4522—an HMG-CoA reductase inhibitor free of metabolically mediated drug interactions: metabolic studies in human in vitro systems [abstract]. J Clin Pharmacol 2000;40:1055.
19. Martin PD, Warwick MJ, Dane AL, Brindley C, Short T. Absolute oral bioavailability of rosuvastatin in healthy white adult male volunteers. Clin Ther 2003;25:2553-63.
20. Martin PD, Mitchell PD, Schneck DW. Pharmacodynamic effects and pharmacokinetics of a new HMG-CoA

reductase inhibitor, rosuvastatin, after morning or evening administration in healthy volunteers. Br J Clin Pharmacol 2002;54:472-7.
21. Hull CK, Penman AD, Smith CK, Martin PD. Quantification of rosuvastatin in human plasma by automated solid-phase extraction using tandem mass spectrometric detection. J Chromatogr B Analyt Technol Biomed Life Sci 2002;772:219-28.
22. Myers GL, Cooper CR, Winn CL, Smith SJ. The Centers for Disease Control—National Heart, Lung, and Blood Institute Lipid Standardization Program: an approach to accurate and precise lipid measurements. Clin Lab Med 1989;9:105-35.
23. Nezasa K, Higaki K, Takeuchi M, Nakano M, Koike M. Uptake of rosuvastatin by isolated rat hepatocytes: comparison with pravastatin. Xenobiotica 2003;33:379-88.
24. Martin PD, Dane AL, Nwose OM, Schneck DW, Warwick MJ. No effect of age or gender on the pharmacokinetics of rosuvastatin: a new HMG-CoA reductase inhibitor. J Clin Pharmacol 2002;42:1116-21.
25. Stapf V, Thalhammer T, Huber-Huber R, Felberbauer F, Gajdzik L, Graf J. Inhibition of rhodamine 123 secretion by cyclosporin A as a model of P-glycoprotein mediated transport in liver. Anticancer Res 1994;14:581-5.
26. Hebert MF. Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. Adv Drug Deliv Rev 1997;27: 201-14.
27. Huang L, Zalikowski J, Dudley A, Grimm S, Wang Y. Investigation of P-glycoprotein-mediated transport of rosuvastatin acid and its lactone across MDR1-MDCK cell monolayers. Presented as a poster at the annual meeting of the American Association of Pharmaceutical Scientists; 2003 Oct 26-30; Salt Lake City, Utah.
28. Cooper KJ, Martin PD, Dane AL, Warwick MJ, Schneck DW, Cantarini MV. Effect of itraconazole on the pharmacokinetics of rosuvastatin. Clin Pharmacol Ther 2003; 73:322-9.
29. Martin PD, Kemp J, Dane AL, Warwick MJ, Schneck DW. No effect of rosuvastatin on the pharmacokinetics of digoxin in healthy volunteers. J Clin Pharmacol 2002; 42:1352-7.
30. Cooper KJ, Martin PD, Dane AL, Warwick MJ, Schneck DW. Lack of effect of ketoconazole on the pharmacokinetics of rosuvastatin. Br J Clin Pharmacol 2003;55:94-9.
31. Yamazaki M, Akiyama S, Ni'inuma K, Nishigaki R, Sugiyama Y. Biliary excretion of pravastatin in rats: contribution of the excretion pathway mediated by canalicular multispecific organic anion transporter. Drug Metab Dispos 1997;25:1123-9.
32. Cooper KJ, Martin PD, Dane AL, Warwick MJ, Raza A, Schneck DW. The effect of erythromycin on the pharmacokinetics of rosuvastatin. Eur J Clin Pharmacol 2003; 59:51-6.