The Effect of a High-Fat Meal on the Oral Bioavailability of the Immunosuppressant Sirolimus (Rapamycin)

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The bioavailability of an oral nonaqueous solution of sirolimus was compared under fasting conditions and after a high-fat meal in a randomized, two-way crossover pharmacokinetic study. Healthy volunteers were administered a 15 mg single dose of sirolimus on two occasions, once while fasting and once after consumption of a high-fat breakfast. Whole blood concentrations of sirolimus were assayed by using a validated method with high-performance liquid chromatography/tandem mass spectrometric detection. Sirolimus was absorbed more slowly when administered after a high-fat meal than when administered after fasting, as shown by statistically significant reductions in peak concentration (C_{max}) and the ratio of C_{max} to the area under the curve (AUC), and lengthening of the time to peak concentration. The oral availability of sirolimus was increased to a modest extent (35%) and in a uniform manner when administered with a high-fat meal; the geometric mean ratio of the fed/fasting AUC values was 1.35, with a 90% confidence interval of 1.26 to 1.46. Food had no effect on the terminal half-life of sirolimus (mean values of 67 to 68 hours). The 35% increase in AUC obtained after a high-fat meal appears small relative to the intersubject and intrasubject variabilities observed in clinical trials. However, to minimize unnecessary fluctuations in trough whole blood sirolimus concentrations, it is advisable that sirolimus be administered consistently in individual patients, either with or without meals.

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Sirolimus (rapamycin, Rapamune®) is a macrocylic triene antibiotic of high molecular weight (913.5 daltons). It was originally isolated from soil samples collected on Easter Island (Rapa Nui) during a screening program for compounds with antifungal or antimicrobial properties. At that time, sirolimus was identified as having significant activity against *Candida* species.[1] It was subsequently discovered that sirolimus is structurally similar to the immunosuppressant drug tacrolimus, that it possesses immunosuppressive properties in vitro, and that it is a potent inhibitor of allograft rejection in animal models.[2-5] Sirolimus is currently under clinical development for prevention of allograft rejection in patients receiving kidney transplants.

Sirolimus is very similar chemically to tacrolimus, and it binds to the same immunophilin binding protein. However, both the mechanism of immunosuppressive action and the side effect profile of sirolimus are distinctly different from those of tacrolimus and cyclosporine (CsA).[6-8] The differences in the mechanism of immunosuppressive action most likely explain why sirolimus and CsA act synergistically to prevent rejection of renal allografts.

The pharmacokinetics of sirolimus have been characterized in renal transplant recipients after single-dose[9] and multiple-dose[10] regimens by oral solution. Absorption of an oral dose is rapid, with mean (%CV) peak concentrations occurring at 1.6 (81%) hours and 1.4 (85%) hours after single and multiple doses, respectively. The declining portion of the whole blood sirolimus concentration-time profile is bi- or triexponential in individual patients. Mean (%CV) terminal half-lives of 63 (28%) hours and 62 (26%) hours have

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been observed after single and multiple doses, respectively. Sirolimus undergoes extensive metabolism, and seven oxidative metabolites have been detected in the whole blood of patients with stable renal allografts.[11] Sirolimus is a substrate for the hepatic and intestinal cytochrome P450 3A4 (CYP3A4) isozymes[12,13] and a substrate for the drug efflux pump P-glycoprotein (P-gp).[14] Similarly, the immunosuppressants cyclosporine[15,16] and tacrolimus are substrates for CYP3A4 and P-gp.

It has been shown that a high-fat meal in stable renal transplant patients increased the oral-dose AUC of cyclosporine from Sandimmune® by 26%.[17] A comparison of the affect of a high-fat meal on cyclosporine dosage forms in renal transplant recipients revealed that the cyclosporine oral-dose AUC was further increased by 22% after switching from Sandimmune® to Neoral®.[18] It has been suggested that the increased absorption of cyclosporine may be partially due to an effect of dietary fat on intestinal metabolism.[19] By contrast, a moderate-fat meal in liver transplant patients decreased the oral bioavailability of tacrolimus by 27%. The purpose of the pharmacokinetic study described in this paper was to assess whether administration of sirolimus with a high-fat meal would alter its oral bioavailability.

METHODS

Clinical study. The study was designed as an open-label, randomized, single-dose, two-way crossover comparison of the pharmacokinetics of a single 15 mg dose of an oral solution of sirolimus when administered to healthy subjects after fasting and after eating a high-fat meal. The clinical part of the study was conducted at the Evanston Hospital Clinical Pharmacology Unit (Evanston, IN) according to the precepts of the Declaration of Helsinki (Hong Kong revisions, 1983) and Good Clinical Practice. The protocol was reviewed and approved by the Evanston Hospital Review Board before initiation of the study. Each subject provided written informed consent before entering the study.

Both male and female subjects were considered eligible to participate if they were between 18 and 45 years of age and in good health; however, female subjects must have been without childbearing potential (surgically sterilized at least 6 months prior to study entry). Eligible participants were to be within 10% of their ideal weight for sex, height, and frame size according to the 1983 Metropolitan Height and Weight Tables. They were confirmed to be healthy by the results of physical examinations, vital signs, and clinical laboratory tests performed during the screening period.

Subjects were ineligible for enrollment if they had a history or evidence of significant cardiovascular, endocrine, gastrointestinal, hematologic, hepatic, neurological, renal, or respiratory disease. Subjects who used psychoactive drugs, recreational drugs, or prescription drugs within 30 days of study drug administration or who had a positive result in the urine screen for drugs of potential abuse were excluded from the study. In addition, subjects could not participate if they had a known hypersensitivity to macrolide compounds such as azithromycin, clarithromycin, and erythromycin or if they had any surgical or medical condition (active or chronic) that might have interfered with the absorption, disposition, metabolism, or elimination of the study drug. Subjects who had any acute illness, including respiratory tract infection, within 2 weeks of study drug administration could not participate in the study.

The subjects were admitted to the Clinical Pharmacology Unit on the day before dose administration on each of the two treatment periods. The two dose administrations were separated by a washout period of 3 weeks or longer. After an overnight fast, each subject either ate a high-fat breakfast over a 20-minute period or continued to fast, as determined by a randomization schedule. The breakfast consisted of two eggs fried in butter, two pieces of bacon, two pieces of toast with butter, 4 ounces of hashed brown potatoes cooked in butter, and 8 ounces of whole milk. The sirolimus dose was administered to the subjects who received the high-fat breakfast within 10 minutes of finishing their meal. At approximately 8:00 a.m., each subject was administered 15 mg/3 mL of sirolimus formulated in a nonaqueous oral solution together with 240 mL of room temperature tap water. No other food or water was given until 4 hours after dose administration, when a standardized lunch was served to all subjects.

Two venous blood samples (5 mL) were obtained before (0, predose) and at 0.33, 0.67, 1, 2, 3, 4, 5, 8, 12, 18, 24, 48, 72, 96, 120, and 144 hours after dose administration. All samples were collected into tubes containing sodium ethylenediaminetetracetic acid (EDTA) and were inverted four or five times. One of each pair of samples was transferred directly into a plastic tube. The other sample of each pair was centrifuged, and the plasma was transferred to a plastic tube. All samples were stored frozen at –70°C until analyzed for blood or plasma concentrations of sirolimus.

Bioanalysis. Sirolimus was assayed in whole blood and plasma samples by using a validated method at Wyeth-Ayerst Research in Princeton, New Jersey. This

method employed high-performance liquid chromatography with tandem mass spectrometry (LC/MS/MS), similar to a previously described method. Modifications were made to eliminate interference from a potential metabolite, secorapamycin.

An internal standard was made by adding 10 ng of 32-desmethoxyrapamycin to a 1 mL sample of whole blood or plasma. The sample was extracted with 7 mL of n-chlorobutane, the organic phase was evaporated to dryness, and the residue was reconstituted with 0.1 mL of methanol/water (70/30, v/v). A 20 µL aliquot of reconstituted sample was injected onto a BDS Hypersil C18 (150 × 2 mm ID, 5 µm) analytical column with a 10 × 2 mm guard column and a small-bore in-line filter (Keystone Scientific). Chromatography was performed at 40°C using a mobile phase consisting of methanol/5 mM ammonium (80/20, v/v) at a flow rate of 0.35 mL/min. A post column splitter was used to divert part of chromatographic elute to the electrospray interface and mass spectrometer. Ions corresponding to m/z ratios of 864.5 and 834.5 were used for the quantitation of sirolimus and internal standard, respectively. The standard curve was calculated by weighted ($1/concentration^2$) linear regression of the peak area ratio of sirolimus/internal standard versus sirolimus concentration.

Using a 1 mL sample, standard curves for sirolimus were linear over the range from 0.10 to 50 ng/mL in both whole blood and plasma. The lowest quantifiable concentrations of sirolimus were 0.10 ng/mL for both blood and plasma. No interfering peaks were observed in blank samples of either matrix. Quality control (QC) samples of 0.3, 5, and 40 ng/mL in blood or plasma showed mean values of percentage error (bias) that were ≤ 7.9%. Between-day coefficients of variation were ≤ 10% for sirolimus concentrations of 5 or 40 ng/mL and ≤ 20% at a concentration of 0.3 ng/mL in both matrices.

Pharmacokinetic analyses. Pharmacokinetic parameters of sirolimus in whole blood were determined by using noncompartmental methods. Peak concentration (C_{max}) and the time of peak concentration (t_{max}) of sirolimus were read directly from the concentration-time profile. Linear regression was used to determine the slope (λ_z) of 4 to 7 points judged to be in the terminal linear phase of the blood concentration-time profile. The terminal half-life ($t_{1/2}$) was calculated as $-0.693/\lambda_z$. Area under the concentration-time profile (AUC_{0-t}) and area under the first moment curve ($AUMC_{0-t}$) were calculated up to the last measurable concentration (C_t) by using the spline method.[20] AUC was extrapolated to infinity by using the formula $AUC_{0-\infty} = AUC_{0-t} + C_t/\lambda_z$. Apparent oral dose clearance (CL/F) was calculated as dose/$AUC_{0-\infty}$. The $C_{max}/AUC_{0-\infty}$ ratio was calculated as an indicator of the absorption rate since the parameter C_{max} is influenced not only by the rate of absorption but also by its extent. Because of the sparseness in available plasma sirolimus concentrations, estimation of sirolimus parameters in plasma was limited to the whole B/P ratios.

Statistical analyses. Statistical analyses were performed by using Statistical Analysis System (SAS, Cary, NC) statistical software.[21] Statistical significance was concluded for a two-sided α value ≤ 0.05. Following log transformation, pharmacokinetic parameters were compared by using two-way analysis of variance (ANOVA) for a two-treatment, two-period, two-sequence design. The factors included in the model were treatment (fasting vs. fed), period, sequence, and subject nested within sequence. The significance of subject, treatment, and period effects was tested by using the mean square for error in the denominator, and the significance of the sequence effect was tested by using the mean square for subject (sequence).

Log-transformed parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, t_{max}, and $C_{max}/AUC_{0-\infty}$) of sirolimus administered to subjects in the fed condition were compared with those in the fasted condition as reference by using the two one-sided tests procedure.[22] Confidence limits were calculated by using least square mean values and the mean square error term from the two-way ANOVA. Bioequivalence was concluded for a parameter if the geometric least square (GLS) mean of the fed/fasted ratio and the 90% confidence interval (CI) around that ratio were within the equivalence range of 0.80 to 1.25.[23]

Safety assessments. Safety was assessed by reports of adverse events and results of routine laboratory evaluations, physical examinations, and electrocardiograms (ECGs). Blood samples for laboratory tests were obtained, and physical examinations were performed at screening and on days 3 and 7 of each period. Complete vital signs (blood pressure, pulse rate, respiratory rate, and oral temperature) were measured at the following time points relative to dosing during each period: 0, 0.67, 124, 48, 72, 96, 120, and 144 hours.

RESULTS

Study population. A total of 23 subjects enrolled in the study; 22 completed both study periods and had evaluable pharmacokinetic data. One subject was

Table I Sirolimus Pharmacokinetic Parameters in Whole Blood of 22 Healthy Subjects Administered a 15 mg Single Oral Dose of Sirolimus after Fasting or after Eating a High-Fat Meal

Parameter (units)	After Fasting	After a High-Fat Meal	*p*-Value[a]
C_{max} (ng/mL)	67.4 ± 22.8	44.4 ± 15.5	0.0001
t_{max} (h)	0.81 ± 0.17	3.08 ± 1.18	0.0001
AUC_{0-t} (ng•h/mL)	635 ± 225	847 ± 273	0.0001
$AUC_{0-\infty}$ (ng•h/mL)	768 ± 272	1027 ± 335	0.0001
$C_{max}/AUC_{0-\infty}$ (/h)	0.098 ± 0.054	0.045 ± 0.012	0.0001
$t_{1/2z}$ (h)	68.3 ± 9.3	66.5 ± 13.1	0.22
CL/F (mL/h/kg)	323 ± 175	229 ± 92	NA

Values are mean ± standard deviation. C_{max}, peak concentration; t_{max}, time of peak concentration; AUC_{0-t}, area under the curve from zero to the last quantifiable concentration; $AUC_{0-\infty}$, area under the curve from zero to infinity; CL/F, apparent oral dose clearance; $t_{1/2z}$, terminal half-life; NA, not assessed.
a. *p*-value for comparison of nonfasting with fasting conditions by using ANOVA for a two-sequence, two-treatment, two-period crossover study.

withdrawn from the study following completion of period 1 (fed condition) because of an adverse event; the data from this subject were included in the safety but not in the pharmacokinetic analyses.

The study population consisted of 21 men and 2 women, who ranged in age from 19 to 43 years (mean = 26.2 years) and in weight from 60 to 102 kg (mean = 71.5 kg). Fifteen of the subjects were white, 4 were black, 3 were Hispanic, and 1 was Asian.

Pharmacokinetics. Whole blood concentrations of sirolimus were above the limit of quantitation through 144 hours (6 days) after dosing in all but 1 subject during one period. The concentrations of sirolimus in plasma generally paralleled those in whole blood. However, sirolimus was quantifiable in plasma for only 3 to 8 hours after dosing in most of the subjects' profiles. Based on ANOVA, there was no significant difference between the fasted and nonfasted conditions with regard to the B/P ratio (data not shown).

Mean concentration-time profiles in whole blood for the entire 144-hour time course following administration of sirolimus to the subjects in the fasted and nonfasted conditions are shown in Figure 1 (panel A). Abbreviated mean profiles in blood for the first 8 hours after dosing are shown in Figure 1 (panel B). The pharmacokinetic parameters derived from the whole blood profiles are summarized in Table I.

The rate at which sirolimus was absorbed was slower in the presence of food, as shown by a significant lengthening of the time to attain peak plasma concentrations together with a significant reduction in C_{max} (Figure 1, panel B; Table I). The C_{max}/AUC ratio was also decreased when sirolimus was administered following a high-fat meal (Table I). Coadministration with food did not appear to alter the appearance of the descending phases of the blood concentration-time

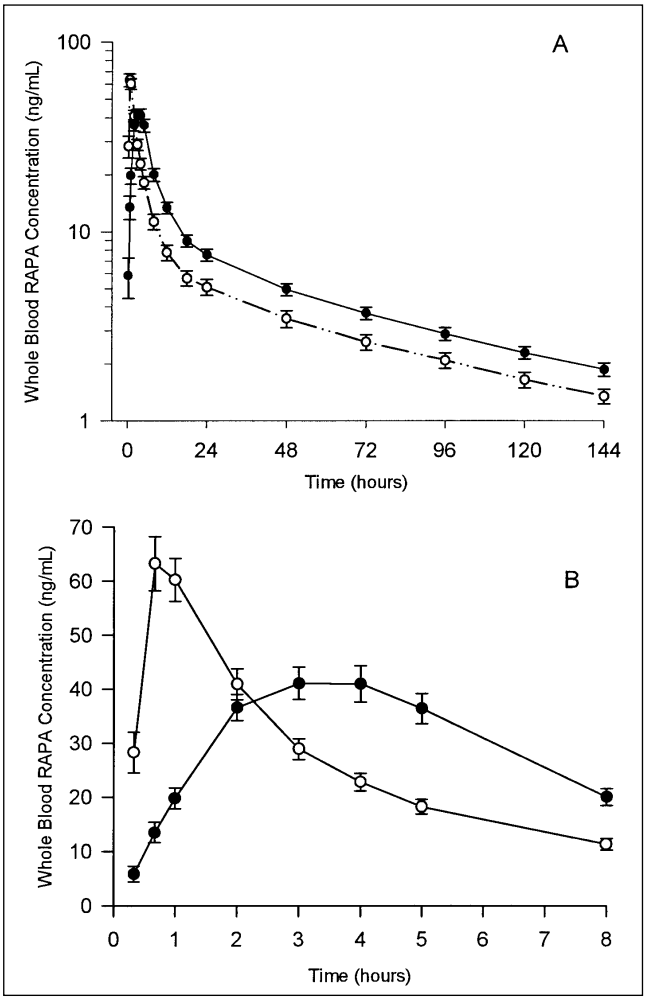

Figure 1. Time course of mean concentrations of sirolimus in whole blood following oral administration of a single 15 mg dose of sirolimus to 22 healthy subjects after fasting (○)or immediately after consuming a high-fat breakfast (●). Panels A and B show the time course for the entire 144 hours and an abbreviated time course for the first 8 hours, respectively.

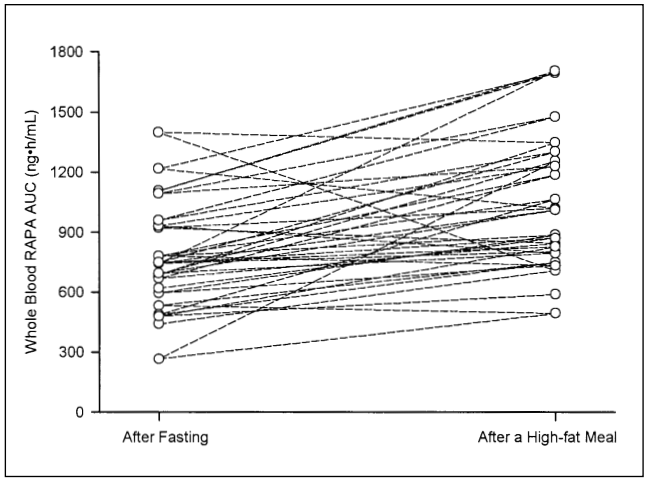

Figure 2. Comparison of AUC values for sirolimus in whole blood of 22 healthy subjects when administered a single oral dose of 15 mg of sirolimus after fasting and after eating a high-fat meal.

Table II Bioequivalence of Whole Blood Sirolimus Pharmacokinetic Parameters after Eating a High-Fat Meal and after Fasting in 22 Healthy Subjects

Parameter	GLS Mean Ratio[a]	90% CI	Bioequivalence Conclusion[b]
C_{max}	0.66	0.61 to 0.71	No
t_{max}	3.54	2.97 to 4.22	No
AUC_{0-t}	1.35	1.26 to 1.45	No
$AUC_{0-\infty}$	1.35	1.26 to 1.46	No
$C_{max}/AUC_{0-\infty}$	0.48	0.45 to 0.53	No

GLS, geometric least square mean; CI, confidence interval; C_{max}, peak concentration; t_{max}, time of peak concentration; AUC_{0-t}, area under the curve from zero to the last quantifiable concentration; $AUC_{0-\infty}$, area under the curve from zero to infinity.

a. GLS mean for ratio of parameter after eating a high-fat meal compared with after fasting as reference.

b. Bioequivalence was concluded if GLS mean ratio and 90% confidence limits around this ratio were contained within the interval of 0.80 to 1.25.

profile (Figure 1, panel A) and had no statistically significant effect on the $t_{1/2}$ of sirolimus (Table I). However, administering sirolimus following a high-fat meal yielded a statistically significant increase in the blood AUC (Table I), indicating an increase in the extent of bioavailability of sirolimus. The magnitude of the increase in the blood AUC of sirolimus after a high-fat meal was modest, and it appeared to be reasonably uniform among individual subjects (Figure 2).

Analysis using the two one-sided test procedure showed that none of the parameters for the rate and extent of sirolimus bioavailability (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, t_{max}, and $C_{max}/AUC_{0-\infty}$) met the criteria for bioequivalence between the fasted and nonfasted conditions (Table II). Following a high-fat meal, the rate of absorption was slowed and the extent of availability of sirolimus was increased relative to the fasting condition. The GLS mean value for the fasted/nonfasted $AUC_{0-\infty}$ ratio was 1.35, indicating an average increase in AUC of 35% when sirolimus was administered in the nonfasted condition relative to fasting. The 90% CI of 1.26 to 1.46 around the $AUC_{0-\infty}$ ratio was narrow.

Safety. One subject was withdrawn from the study because he developed a right lower-lobe pneumonia between periods 1 and 2. The investigator considered this adverse event to be moderate in severity and possibly related to study drug administration. The subject's pneumonia resolved completely following a 10-day course of oral erythromycin.

Sixteen (69%) of the 23 subjects experienced one or more adverse events during the study. A total of 51 adverse events were reported; 24 were reported by 10 subjects during the nonfasted arm, and 27 were reported by 11 subjects during the fasted arm of the crossover. All adverse events were mild or moderate in severity, and none was considered to be serious by the investigator. The most frequent complaints were headache (5 subjects), pharyngitis (4 subjects), rhinitis (4 subjects), and urinary frequency (3 subjects).

Two abnormal laboratory results observed subsequent to randomization were reported as adverse events. One subject experienced a transient increase in serum potassium (normal range, 3.6-5.5 mmol/L) from 4.6 mmol/L at baseline to 5.7 mmol/L on day 3 of period 2 (fed condition). Additional serum samples obtained from this subject on days 4 and 7 of this period showed potassium values that were within the normal range (5.0 and 4.6 mmol/L, respectively). The investigator considered this episode of hyperkalemia to be mild in severity and possibly related to study medication. A second subject had mild hematuria on day 28 after administration of sirolimus with a high-fat meal; this occurrence was considered not related to study medication by the investigator. No subject had any clinically important changes in vital signs or physical examination results.

DISCUSSION

Sirolimus is similar to tacrolimus in chemical structure, and these two compounds also resemble each other with respect to some pharmaceutical and pharmacokinetic characteristics. The similarities include high lipophilicity, very low solubility in aqueous media,[24,25] and high B/P ratio.[9,10,25]

It has been stressed that a priori prediction of the direction and magnitude of an effect of food on the bioavailability of a drug can be difficult.[26,27] This study showed that administration of sirolimus following a high-fat meal slows the rate of absorption and modestly increases the extent of systemic bioavailability from a nonaqueous oral formulation. In the case of tacrolimus, a 71% mean decrease in C_{max} and a 39% mean decrease in AUC_{0-t} was observed when the marketed capsule formulation was administered immediately following a high-fat meal relative to when it was administered in the fasting state.

A variety of mechanisms have been invoked to explain an increase in the extent of oral availability when a compound is administered with food.[26-32] Food-induced delays in gastric emptying and a slower input rate into the proximal intestine may prevent saturation of absorption mechanisms, particularly if there appears to be a local absorption window. Both dietary fats and food-stimulated secretions such as bile salts may facilitate solubilization and dispersion, particularly of lipophilic compounds. Increases in sphlanchnic blood flow and competition by food components for metabolic enzymes may reduce the extent of first-pass metabolism.

In the case of sirolimus, food did not alter elimination, as shown by the absence of an effect on the terminal disposition half-life (Table I) and the strikingly similar postabsorption profiles in the presence and absence of food (Figure 1, panel A). The 35% increase in systemic availability of sirolimus was probably due to some combination of facilitated absorption and inhibition of CYP3A4 and P-gp in the intestine.

The safety profile of sirolimus in this study of healthy subjects was not markedly different from that reported from studies in patients with renal transplants.[10,33,34] One subject in this study developed pneumonia, which led to his premature withdrawal; however, it seems unlikely that a single dose of sirolimus would cause a degree of immunosuppression sufficient to increase his susceptibility to infection.

The results of the current study may not appear to be directly relevant to sirolimus administration in renal allograft recipients. First, the study was designed to simulate the most extreme effect of food (i.e., high-fat meal) on the bioavailability of sirolimus. However, high-fat meals would not be recommended for transplant patients because the vast majority of such patients are hyperlipemic and/or hypercholesterolemic. Second, it may not be practical to administer sirolimus in the fasting state during cotherapy with CsA. Based on the results of pivotal phase III trials, sirolimus should be administered 4 hours after the morning dose of CsA, a time point that would most likely coincide with the midday meal. Third, experience in clinical trials has revealed large inter- and intrasubject variabilities in trough whole blood sirolimus concentrations. In the current study, the range of CL/F values among subjects was 7-fold in the fasted state (136 to 959 mL/h/kg) and 4.2-fold after a high-fat meal (121 to 509 mL/h/kg). The 35% increase in AUC obtained after a high-fat meal appears small in relation to such variabilities. However, to minimize unnecessary fluctuations in trough whole blood sirolimus concentrations, it is advisable that sirolimus be administered consistently in individual patients, either with or without meals.

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