Population pharmacokinetics of sirolimus in kidney transplant patients

Objective: To characterize the dose-related pharmacokinetics of the immunosuppressant agent sirolimus (formerly rapamycin) in kidney transplant patients by use of two-stage and nonlinear mixed-effect model population methods.

Methods: Patients ($n = 36$) from three centers (Germany, the United Kingdom, and Sweden) who received steady-state oral doses of cyclosporine (ciclosporin) were assessed after single oral administration of sirolimus at doses of 3, 5, 10, and 15 mg/m^2. Plasma and whole blood sirolimus samples were analyzed by a high-performance liquid chromatographic/mass spectrophotometric method. Simultaneous fitting used biexponential functions with intercept/slope or clearance/volume terms, as well as first-order absorption (k_a) and a lag-time.

Results: The nonlinear mixed-effect model method (P-Pharm) provided a better characterization of sirolimus kinetics, especially for the absorption and distribution phases where fewer data were available per patient. Sirolimus distribution between whole blood and plasma was concentration-independent, with a mean blood/plasma ratio (coefficient of variation) of 30.9 (48.5%). Elimination was not influenced by dose, as shown by estimates of the terminal half-life of 63 hours (27.5%) and apparent oral blood clearance of 8.9 L/hr (38.2%). Sirolimus distribution parameters were influenced by body weight and surface area. Sirolimus was rapidly absorbed, as shown by the absorption lag-time of 0.27 hour (35.1%), and k_a of 2.77 hr^{-1} (48.4%). The concomitant administration of sirolimus and cyclosporine did not reveal any pharmacokinetic interactions.

Conclusion: This report provides an initial population pharmacokinetics of sirolimus in kidney transplant recipients receiving cyclosporine concurrently. Sirolimus blood and plasma pharmacokinetics were biexponential and linear for doses from 3 to 15 mg/m^2. No pharmacokinetic interaction was found between sirolimus and cyclosporine. (Clin Pharmacol Ther 1997;61:416-28.)

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Sirolimus (formerly rapamycin) is an immunosuppressant[1] that is effective for the prevention of allograft rejection in experimental animals.[2] The drug is more potent in vitro than cyclosporine (ciclosporin),[3,4] and its cellular mechanism of action is different. Sirolimus has been shown to inhibit T-cell activation through suppression of interleukin-2 (IL-2) and interleukin-4 (IL-4) driven T-cell proliferation, whereas cyclosporine inhibits T-cell stimulation by reduction of IL-2 production and interleukin receptor (IL-2R) expression.[2,5-7] In vitro and in vivo studies have shown the synergistic interaction of the two drugs.[8,9] The pharmacokinetics of cyclosporine is well characterized in humans,[10] whereas sirolimus disposition has been described preliminary in one group of patients.[11] Because the chemical structure and properties of sirolimus are similar to those of tacrolimus and cyclosporine, sirolimus human disposition could be expected to include a large volume of distribution related to high tissue and red blood cell partitioning, extensive liver metabolism, intermediate to a long elimination half-life, and low oral bioavailability.[10,12,13]

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To optimize the clinical use and to minimize the risk of adverse events, pharmacokinetic studies of drugs must evaluate not only average population pharmacokinetic parameters but also their interpatient variability. In addition, factors of demographic (age and weight), environmental (food and study site), or drug-related (e.g., cyclosporine) origin that may influence patient exposure to the drug should be identified.[14,15] For these purposes, two analysis methods are commonly used: the two-stage method, which is the study of covariable influences on parameters calculated by individual modeling; and the nonlinear mixed-effect model method (NMEM). The NMEM approach is known to give better characterization of the interpatient variability in parameter values; the two-stage method usually overestimates the interpatient variabilities because of less accurate parameter estimation in phases where few data are available for each individual.

This study was designed to provide the population pharmacokinetic characteristics of sirolimus in patients with stable kidney transplants and to examine the effect of dose (3 to 15 mg/m^2) on these characteristics. The mean and interpatient variability of each kinetic parameter for the absorption, distribution, and elimination phases were calculated by use of the two-stage and NMEM methods. Initially, a polyexponential function was used to describe the time course of both plasma and blood concentrations simultaneously. The resulting joint intercept and slope parameters were changed to clearance and volume terms to make the population kinetic model more physiologically relevant. The influences of demographic (study center, age, and weight) and drug-related (cyclosporine administration) factors on the interpatient variability of various pharmacokinetic parameters were then assessed.

METHODS

Patient populations

Phase I oral safety and tolerance assessments were carried out in Germany, the United Kingdom, and Sweden with different populations of kidney transplant patients, including patients with a high risk of chronic rejection (Germany), patients with compromised kidney function (United Kingdom), and patients with stable transplants (Sweden).

All patients were receiving steady-state doses of cyclosporine sufficient to maintain therapeutic blood levels. Swedish and British patients also generally received maintenance doses of therapeutic steady-state oral prednisone and azathioprine; however, no other immunosuppressants were administered to the German patients.

Table I. Summary of patients characteristics by study center

	Study center		
	Germany	*United Kingdom*	*Sweden*
Age (yr)	52.3 (24.0)	51.0 (25.5)	43.3 (29.0)
Gender			
Male	11	11	9
Female	1	1	3
Height (cm)	171.8 (4.6)	171.6 (6.7)	172.8 (4.0)
Weight (kg)	73.9 (17.5)	78.2 (28.7)	75.6 (18.9)
Body surface area (m^2)	1.87 (10.1)	1.90 (15.7)	1.88 (10.0)

Data are presented as the mean (SD) (n = 12).

Patients were allowed other concomitant medications, provided that the regimen was initiated at least 2 weeks before sirolimus administration and was continued for the duration of the study. The administration times of these concomitant medications were adjusted to minimize effects on sirolimus pharmacokinetics. The median number of such medications administered were 3 (range, 0 to 7) in Germany, 2 (range, 0 to 6) in the United Kingdom, and 3.5 (range, 0 to 5) in Sweden. Prohibited medications included terfenadine, nonsteroidal antiinflammatory drugs, potassium-sparing diuretics, or cytotoxic agents. Alcoholic or recreational drugs were also not permitted.

The demographic characteristics of the patients are presented in Table I. Very similar values for age, height, weight, and body surface were observed among the three study centers. The overall mean ± SD values for the age, weight, height, and body surface area of the patients among centers were 49 ± 13 years (range, 20 to 68 years), 75.9 ± 16.7 kg (range, 52 to 121.5 kg), 172 ± 9 cm (range, 150 to 188 cm), and 1.89 ± 0.23 m^2 (range, 1.5 to 2.43 m^2). The total number of patients receiving sirolimus was 36 (12 at each center), four of whom were women; none of the patients were of African descent.

Drug formulation

Oral sirolimus (5 mg/ml) was provided in a nonaqueous mixture obtained from Wyeth-Ayerst Research (Radnor, Pa.). The concentrate was protected from light and refrigerated in 5 ml flint-glass

vials that enabled withdrawal of 2.5 ml. The sirolimus dose was determined based on body surface area (mg/m^2). The final solution was prepared in a glass vessel by diluting the appropriate volume of sirolimus concentrate with sterile water and vigorously stirring for at least 1 minute.

Study design

After approval by the appropriate institutional committee at each center, patients were enrolled into a randomized, double-blind, placebo-controlled, ascending single-dose study. Four doses (3, 5, 10, and 15 mg/m^2) were evaluated consecutively at each center, with three patients receiving sirolimus and one patient receiving placebo within each dose group (one patient inadvertently received a dose of 7.8 instead of 5 mg/m^2). On day –1, patients reported to the center in the fasting state at approximately 7 AM and received a light low-fat liquid breakfast. At 1 hour after breakfast, cyclosporine was administered with 240 ml of room temperature distilled water. On day 1, the timing of the breakfast and dose administration were the same as on day –1, except that cyclosporine and sirolimus were administered consecutively, each with 120 ml water. Patients abstained from food for 3 hours after the morning dose administration. All meals had a standardized 30% fat content to minimize the effect of fat on cyclosporine absorption. Patients were discharged from the study center on day 7.

On day –1, whole blood was collected for blood cyclosporine determinations before (0 hours) and at ½, 1, 2, 3, 6, and 12 hours after dose administration. On day 1, blood was collected for blood cyclosporine, plasma sirolimus, and blood sirolimus determinations before (0 hour) and at ½, 1, 2, 3, 6, and 12 hours after dose administration. Blood was collected before cyclosporine dosing for blood cyclosporine, blood sirolimus, and plasma sirolimus determinations on day 2 (trough), except at the British center where the blood was collected on days 3 and 5. Blood samples for plasma sirolimus determinations were rapidly centrifuged at 4° C to separate the plasma. A total of 636 sirolimus whole blood and plasma samples were obtained among the centers, with 13 to 24 sets per patient.

Assay methods

Sirolimus. Blood and plasma samples were analyzed for sirolimus by a validated electrospray high-performance liquid chromatographic/mass spectrophotometric (HPLC/MS) method (TexMS Analytical Services, Houston, Texas). A 1 ml aliquot of whole blood was spiked with desmethoxyrapamycin as the internal standard. The samples were extracted with 5 ml of *n*-butyl chloride. After removal of the organic layer and evaporation to dryness, the residue was reconstituted in 150 μl of 2:1 acetonitrile/ methanol and subjected to solid-phase extraction by use of a C_{18} reversed-phase column (LC-Packings, San Francisco, Calif.). The eluant was dried under nitrogen and reconstituted in 20 μl methanol. A 1 μl aliquot was analyzed by electrospray HPLC/MS (Mermag, model R-110). The ratio of the intensities (*m/z* 937)/(*m/z* 907) for sirolimus and desmethoxyrapamycin was used to calculate the concentration of sirolimus by use of a calibration curve. This was generated from the analysis of a drug-free whole blood matrix fortified with various amounts of sirolimus and a fixed amount of the internal standard. The methods for blood and plasma analysis were linear over the ranges from 0.25 to 50 ng/ml for blood and 0.25 to 10 ng/ml for plasma, with a 1 ml sample.

Assay performance during sample processing was assessed by precision and accuracy of the standard concentrations used for calibration curves and the quality control samples. Precision was expressed as the coefficient of variation (CV) at each concentration. Accuracy was measured as the percent deviation of the observed from the theoretical concentrations. For blood calibration standards (0.25, 0.5, 1, 5, 10, 25, and 50 ng/ml), the mean interday CV values for precision ranged from 2.1% to 8.9%, and accuracies expressed as a mean percentage error ranged from –0.8% to 3.2%. For blood quality control standards (0.75, 15, and 40 ng/ml), the mean values of the interday CV for precision ranged from 6.6% to 8.2%, and accuracies ranged from –0.5% to 1.3%. For plasma calibration standards (0.25, 0.5, 1, 5, 10, and 20 ng/ml), the mean values of the interday CV for precision ranged from 3.2% to 5.1%, and accuracies ranged from –0.6% to 1.3%. For plasma quality control standards (0.70, 2.5, and 8 ng/ml), the mean CV values for precision ranged from 7.8% to 8.4%, and accuracies ranged from 0.2% to 0.6%.

Sirolimus concentrations below the limit of quantification (0.25 ng/ml) were not used in the two-stage analysis. However, in the NMEM analysis, all whole blood and plasma sirolimus concentrations greater than 0.25 ng/ml were entered as "reliable" data (status = 0) and concentrations between 0.125 and 0.25 ng/ml were considered "uncertain" data (status = 1); the P-Pharm software (SIMED, Creteil,

France) assigned a lower weight to the uncertain data.

Cyclosporine. Whole blood samples were analyzed for cyclosporine by a validated radioimmunoassay with a specific anti-cyclosporine monoclonal antibody (Cyclo-Trac SP-Whole Blood, INCSTAR Corporation, Stillwater, Minn.). The unbound fraction of [^{3}H]-cyclosporine resulting from competitive interaction with unlabeled cyclosporine was quantitated by beta liquid scintillation counting. The method was linear over 25 to 1600 ng/ml with 1 ml of blood. The interrun CV values ranged from 2.4% (150 ng/ml quality control) to 6.8% (60 ng/ml quality control). Assays were conducted by Addenbrooke's Hospital, Cambridge, England.

Pharmacokinetic model

Paired whole blood and plasma sirolimus concentration versus time data were analyzed by use of both PCNONLIN (SCI Software Inc, Apex, N.C.) and P-Pharm; plasma concentrations were related to whole blood concentrations by the whole blood to plasma ratio (B/P). The first simultaneous fitting model used a biexponential function with first-order absorption and lag-time (t_{lag}) to obtain an estimation of the intercept coefficients (A and B), the slopes of the distribution (α) and elimination (β) phases, the absorption rate constant (k_a), t_{lag}, as well as the ratio (blood/plasma) between concentrations in whole blood (C_b) and plasma (C_p):

$$C_b = \text{dose} \cdot (A \cdot e^{-\alpha \cdot (t - t_{lag})} + B \cdot e^{-\beta \cdot (t - t_{lag})} - (A + B) \cdot e^{-k_a \cdot (t - t_{lag})}) \quad (1)$$

$$C_p = C_b/(B/P) \quad (2)$$

For triexponential fittings, (A+B) was changed to (A+B+C) and a third term ($C \cdot e^{-\gamma \cdot (t - t_{lag})}$) was added to equation 1. The discrimination between the biexponential and triexponential models was made by use of either graphical (estimated versus observed concentrations) or mathematical (Akaike information criterion, maximum likelihood function value) comparisons.

To have a more physiologically relevant model, apparent elimination clearance (CL), distribution clearance (CL_d), and volume terms (central [V_c] and peripheral volume [V_p]) were introduced in equation 1. Thus clearance and volume terms replaced intercept and slope parameters. The interrelationships between these coefficients and parameters are as follows:

$$\alpha = 0.5 \cdot \left[\frac{CL_d}{V_c} + \frac{CL_d}{V_p} + \frac{CL}{V_c} + \sqrt{\left(\left[\frac{CL_d}{V_c} + \frac{CL_d}{V_p} + \frac{CL}{V_c}\right]^2 - 4 \cdot \frac{CL_d \cdot CL}{V_c \cdot V_p}\right)}\right] \quad (3)$$

$$\beta = 0.5 \cdot \left[\frac{CL_d}{V_c} + \frac{CL_d}{V_p} + \frac{CL}{V_c} - \sqrt{\left(\left[\frac{CL_d}{V_c} + \frac{CL_d}{V_p} + \frac{CL}{V_c}\right]^2 - 4 \cdot \frac{CL_d \cdot CL}{V_c \cdot V_p}\right)}\right] \quad (4)$$

$$A = \frac{k_a}{V_c} \cdot \frac{(CL_d/V_p - \alpha)}{(k_a - \alpha) \cdot (\beta - \alpha)} \quad (5)$$

$$B = \frac{k_a}{V_c} \cdot \frac{(CL_d/V_p - \beta)}{(k_a - \beta) \cdot (\alpha - \beta)} \quad (6)$$

The different kinetic models were validated by the assessment of individual and population area under the whole blood concentration–time curve (AUC) bias calculated with the equation 7, taking AUC calculated with the noncompartmental method (AUC_{ncpt}, spline method) as the reference. A correct fitting was considered to provide mean and individual bias of less than 15%. The area under the whole blood concentration of sirolimus versus time curve was calculated as $AUC_{pop} = A/\alpha + B/\beta - (A + B)/k_a$ for the polyexponential model and as dose/CL for the semiphysiologic model:

$$\text{Bias}\,(\%) = 100 \cdot \frac{(AUC_{pop} - AUC_{ncpt})}{AUC_{ncpt}} \quad (7)$$

Population pharmacostatistical models

The goal of population pharmacokinetic analysis is to quantify parameter variabilities within the studied population and to explain them by the addition of covariate influences. In other words, for an individual j, the volume of distribution (V_{cj}) can be expressed as a function of the population value (V_{cpop}) plus an interpatient variability term (η_j^{Vc}). Assuming a normal distribution for the volume parameter inside the population (η_j^{Vc} of mean zero, and variance σ^2_{Vc}), equation 8 can be written as follows:

$$V_{cj} = V_{cpop} + \eta_j^{Vc} \quad (8)$$

If the volume of distribution were found to significantly increase linearly with increased weight ($V_{cpop} = a + b \cdot \text{weight}_{pop}$), equation 8 would be transformed to equation 9:

$$V_{cj} = a + b \cdot \text{weight}_j + \eta_j^{Vc} \quad (9)$$

Similar equations can be written for each pharmacokinetic parameter, and the estimated concentration (F_{ij}) at time t_i for individual j is obtained from equation 1. The observed concentration C_{ij} will differ from the estimated by the residual error (ϵ_{ij}), which was assumed to be proportional to C_{ij}. Therefore, C_{ij} can be expressed as equation 10:

$$C_{ij} = F_{ij} \times (1 + \epsilon_{ij}) \quad (10)$$

Two-stage approach

A traditional two-stage procedure was used to assess the population kinetic characteristics. In the first stage, individual fittings with use of PCNONLIN gave the parameter estimates. In the second stage, the individual values were used to provide the parameter distribution and to compute their mean and variance under the assumption of a normal distribution. Independent covariates were directly studied by graphing individual parameter estimates as a function of each covariable (Sigma plot, version 2.0, Jandel Scientific, San Rafael, Calif.), regression analysis (for covariates with a continuous distribution (e.g, weight), or variance analysis for covariables with a discrete distribution: (e.g, dose) (SAS/stat, version 6, SAS Institute Inc., Cary, N.C.).

NMEM method

Nonlinear mixed-effect population modeling was implemented by use of P-Pharm, a new NMEM modeling program for a Windows environment. Population pharmacokinetic parameters were modeled in terms of both random (parameter variance) and fixed effects (mean population parameter and influence of independent variables). Fixed effects account for interpatient differences in the values of independent variables identified as covariables (age and weight), and they help to explain part of the parameter variability: for example, decreased renal clearance is often related to increased age. The influence of the covariable was entered by using a linear function. With the example above for patient j, $CL_j = a + b \cdot age_j$, in which a is the intercept and b is the slope of the linear relationship. Random effects account for unexplained interpatient variability, the distribution of the error terms being normal or log-normal. P-Pharm computes the mean population parameters values together with their variance and CV% with an expected maximization–type algorithm.[16] This CV% does not reflect the precision of the population parameter estimate, but provides the variance about the mean population value (e.g., Eq. 8). This algorithm is an iterative process suitable for computing the maximum likelihood estimates in complicated problems of missing and incomplete data. With use of a bayesian estimator, individual parameters are estimated in the first step (expected step), assuming that the population characteristics are known. The population parameters are then estimated in the second step (maximization step) with use of the maximum likelihood principle and linearization about the bayesian estimates. P-Pharm has similar performances as the NONMEM first-order conditional estimates method within a limited number of iterations ($n < 100$) and a much smaller computation time.[17] Moreover, a multidimensional regression is implemented in P-Pharm to assist during the covariable inclusion step (F test). During the computation of population parameters, a standard least-square algorithm was used: the weight was chosen to be one over the square of the estimated concentration value (variance model with constant coefficient of variation). The comparison between the models was based on goodness-of-fit by graphic (estimated versus observed concentrations for plasma and whole blood) and mathematic (likelihood ratio test) evaluations included in P-Pharm.

During the search for the optimum population pharmacokinetic model, the population and interpatient variability values of each parameter were first computed. Then the variability of each parameter was studied to assess the influence of specific covariables (i.e., age, weight, height, body surface area, dose in milligrams and in milligrams per square meter of body surface area, cyclosporine exposure on day 1, and study site). Both graphic (individual parameter value versus weight, cyclosporine exposure, . . .) and statistical evaluations (multilinear regression) were used. The distribution of all parameters was assumed to be normal at the beginning, and this assumption was checked at the end of the analysis by means of the Kolmogorov-Smirnov test.[18] A search of outlier values for parameters and residuals was performed with the T method.[19] A graphic validation of this statistical analysis was performed with plots of estimated versus observed drug concentrations for plasma and whole blood and individual fittings.

RESULTS

Sirolimus pharmacokinetics

Noncompartmental approach. The dose-normalized blood and plasma concentrations of sirolimus are shown in Fig. 1. A rapid absorption phase was followed by a biexponential decline with a long termi-

nal half-life ($t_{1/2\beta}$) of approximately 60 hours. Both plasma and whole blood concentrations reached a peak (C_{max}) between 1 and 2 hours after sirolimus dose administration and both showed similar decline. At the latest sampling times, few plasma data were available because of limitations in assay sensitivity.

The individual parameter estimates from noncompartmental analysis are shown in Fig. 2. The sirolimus blood/plasma ratio was variable, with a mean of 34.5 (CV, 52.3%; range, 10 to 70); no significant differences were observed between centers or over the dosage range. The mean $t_{1/2\beta}$ was 60.7 hours (CV, 27.5%; range, 36.6 to 109.7 hours). This long and variable $t_{1/2\beta}$ was also dose-independent and similar among study sites. Analysis of the whole blood AUC and C_{max} confirmed their linear relationship with oral doses of 3 to 15 mg/m^2 (r = 0.88 and r = 0.85). Thus, in the range of oral doses studied, whole blood and plasma sirolimus pharmacokinetics were linear.

Population approach with a polyexponential model. Both biexponential (equations 1 and 2) and triexponential functions with first-order absorption and t_{lag} were used in characterizing the sirolimus whole blood and plasma data (Fig. 1). The maximum likelihood and Akaike information criterion values remained constant, indicating that the triexponential equation did not significantly improve the characterization of sirolimus kinetics. The population parameter values obtained from a biexponential model with the two-stage and NMEM approaches are summarized in Table II. Covariate analysis with P-Pharm based on both graphic and statistical associations revealed an influence of body weight on the distribution slope (α). The analysis showed that 43% of the interpatient variability in α was explained by the influence of weight: $\alpha_i = a + b \cdot \text{weight}_i$, in which a = 0.0806 hr^{-1} and b = 0.002674 ± 0.00053 hr^{-1}/kg. Similar results were obtained for the influence of body surface area because weight and body surface are highly correlated (r = 0.97). No influence of dose, study site, cyclosporine, or weight was found for any other parameters.

The P-Pharm population value of the distribution intercept (A) was 7.89 ng/ml, with an interpatient variability of 38.9% (one outlier detected). These values were lower than those estimated by the two-stage analysis (12.22 ng/ml and 95.7%). The same trend was found for the elimination intercept (B). The difference between the two methods appears to relate to better estimation of the absorption phase with P-Pharm.

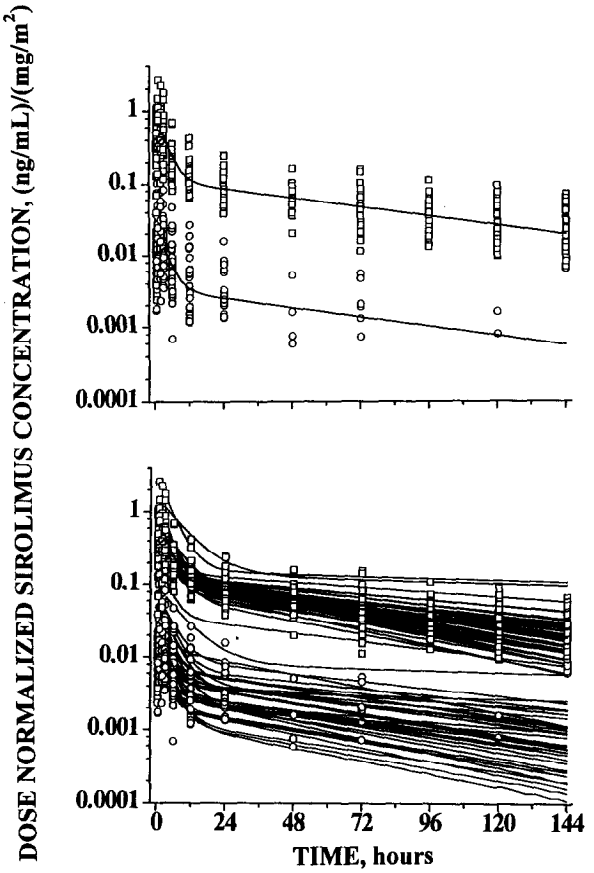

Fig. 1. Sirolimus whole blood *(squares)* and plasma *(circles)* concentrations normalized by the dose as a function of time after single oral administration of doses of 3, 5, 10, and 15 mg/m^2 in 36 kidney transplant patients. Individual **(lower panel)** and population **(upper panel)** fittings were obtained with the complete semiphysiologic model.

For the slope parameters, the P-Pharm population mean and interpatient variability values of α were 0.284 hr^{-1} and 25.1%. These values differed from the two-stage analysis (0.442 hr^{-1} and 63%). The lower CV from NMEM showed that gathering the data increased the precision and accuracy of mean parameter estimations. This was particularly true in characterization of phases for which few data were available for each patient (e.g., the absorption phase). For β, the P-Pharm population mean and interpatient variability were found to be similar to the two-stage estimates. In fact, 4 to 5 data points were obtained in the elimination phase for each patient, allowing good characterization of the terminal slope by either method.

For the absorption parameters, gathering data from different patients increased the information

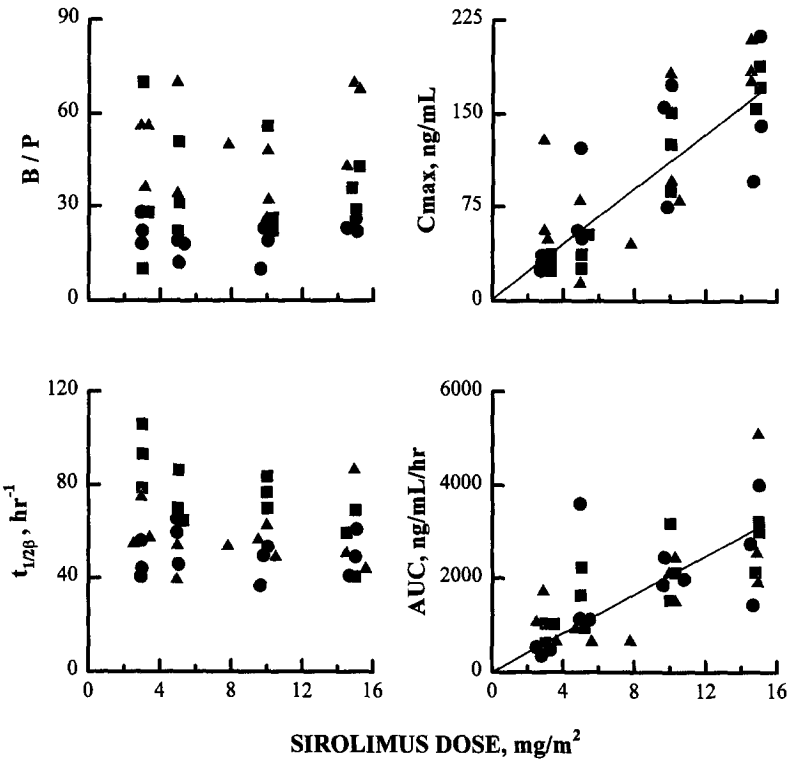

Fig. 2. Relationships between administered dose of sirolimus (mg/m^2) and whole blood to plasma ratio (B/P), maximal blood concentration (C_{max}), elimination half-life ($t_{½\beta}$), and whole blood exposure (AUC) values obtained by noncompartmental analysis. Regression lines are shown for C_{max} and AUC panels. Parameter values are designated by center: Germany *(squares),* United Kingdom *(circles),* and Sweden *(triangles).*

Table II. Mean population parameter estimates obtained with the standard two-stage (PCNONLIN) and the nonlinear mixed-effect model (P-Pharm) methods, with the use of the biexponential function

	Two-stage method		*NMEM method*	
Sirolimus parameters	*Mean*	*Interpatient variability (CV%)*	*Mean*	*Interpatient variability (CV%)*
A (ng/ml)	12.22	95.7	7.89	38.9
B (ng/ml)	1.25	46.4	1.06	35.5
α (hr^{-1})	0.44	63.1	0.28	25.1
β (hr^{-1})	0.013	30.1	0.011	27.5
k_a (hr^{-1})	6.50	93.0	2.77	48.4
t_{lag} (hr)	0.29	51.7	0.27	35.1
B/P ratio	34.4	51.2	30.9	48.5

A and B, Intercept coefficients; α, distribution slope; β, elimination slope; k_a, absorption rate constant; t_{lag}, lag-time; B/P ratio, blood/plasma ratio.

available for fitting this phase. The NMEM mean value of the k_a was 2.77 hr^{-1} (CV, 48.4%; range, 0.45 to 4.14 hr^{-1}), which was lower than the k_a of 6.53 hr^{-1} (CV, 92.9%; range, 0.40 to 24.2 hr^{-1}) obtained with the two-stage method. For the t_{lag}, P-Pharm yielded a mean value of 0.269 hour (CV, 35.1%), whereas the two-stage value was 0.29 hour (CV, 51.7%). No outlier value was found with NMEM, but several unusual values were observed with the two-stage method.

The P-Pharm population mean of the blood/plasma ratio was 30.9 (CV, 48.5%), which was very

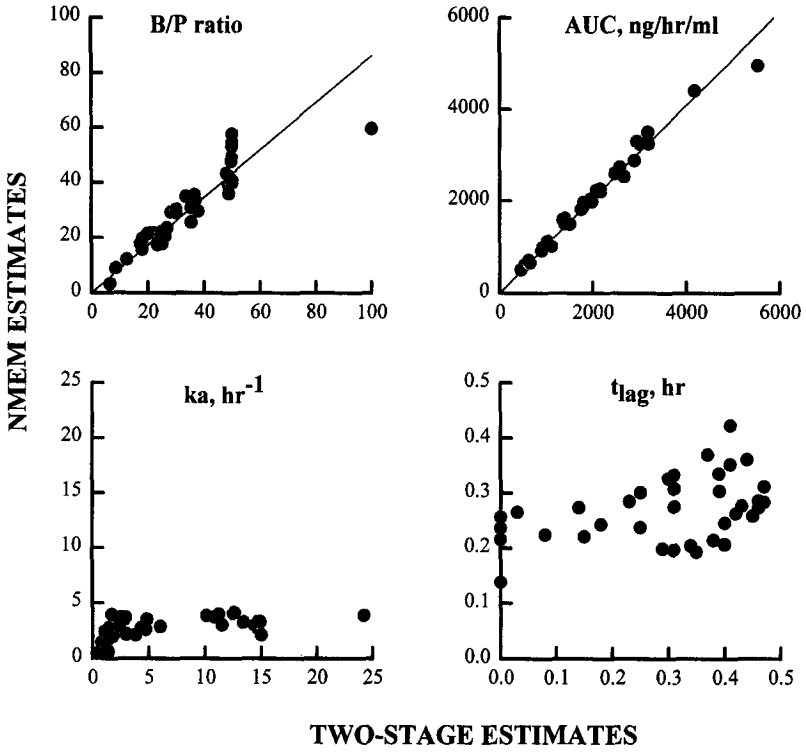

Fig. 3. Comparison of selected pharmacokinetic parameters obtained by the two-stage (PCNONLIN) and NMEM (P-Pharm) methods. k_a, absorption rate constant; t_{lag}, lag-time.

similar to the results of 34.4 (CV, 51.2%) and 34.5 (CV, 52.3%) obtained with the two-stage and noncompartmental analyses, respectively. The close agreement of these results obtained by different approaches may have been related to the ample number of plasma data (>3) available for individual patients.

The validation of sirolimus pharmacokinetic estimates obtained by both population approaches was confirmed by the mean percentage AUC bias ± SD based on equation 7: 2.5 ± 5.6% for P-Pharm and –2.13% ± 5.6% for the two-stage method. Percentage biases in individual patients were no greater than ±15%. Moreover, the close agreement between the AUC values obtained by the two population kinetic methods was confirmed by the slope of 1.02. A graphic comparison of individual parameter estimates for k_a, t_{lag}, the blood/plasma ratio, and AUC obtained with two-stage and P-Pharm is shown in Fig. 3.

Population approach with the semiphysiologic model. A semiphysiologic model based on a biexponential function with first-order absorption and t_{lag} (equations 1 to 6) was used in characterizing the sirolimus whole blood and plasma data (Fig. 1). The resulting population parameter values obtained with the two approaches are summarized in Table III. Individual and population values obtained with P-Pharm are displayed in Fig. 1. Covariate analysis with P-Pharm based on graphic and statistical assessment pointed out an influence of body weight on CL_d and V_p. For CL_d, 48% of the interpatient variability was explained by the influence of weight: $CL_{di} = a + b \cdot weight_i$, in which a = –1.55 L/hr and b = 0.352 ± 0.0625 L/hr/kg. For V_p, 35% of the interpatient variability was related to the influence of weight: $V_{pi} = c + d \cdot weight_i$, c = 141.8 L, and d = 4.09 ± 0.96 L/kg (Fig. 4). Similar results were obtained with body surface area and were in agreement with the influence of weight previously found on the distribution slope. No influence of dose, study site, cyclosporine exposure, or weight was found for any other parameters.

The P-Pharm population mean value of the central volume was 112.9 L, with an interpatient variability of 31.8%. These values were lower than those obtained with the two-stage analysis (129.9 L and

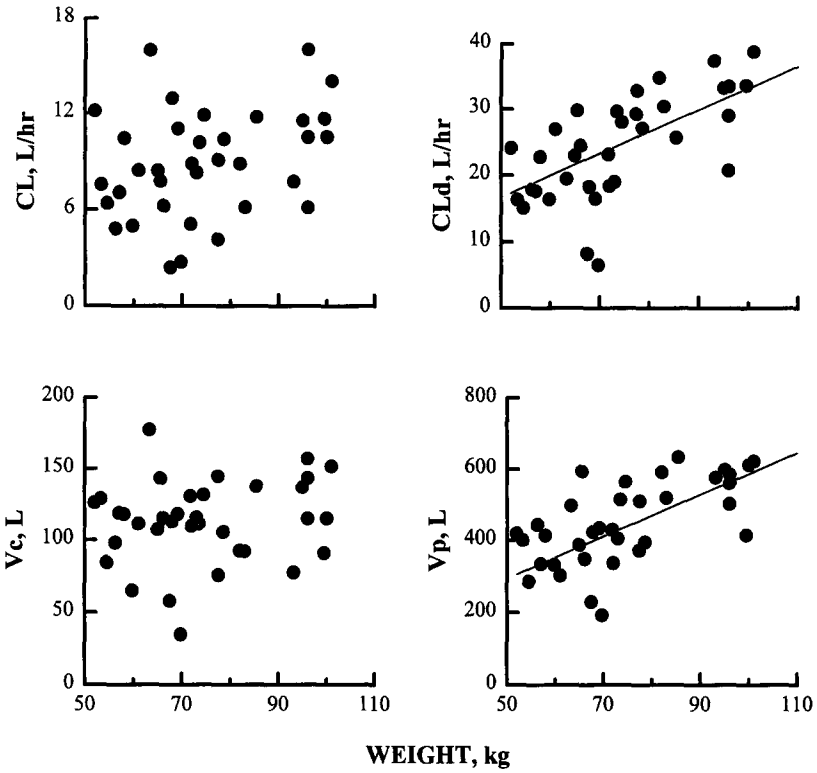

Fig. 4. Influence of body weight on the clearances (elimination clearance [CL] and distribution clearance [CL_d]) and volumes (central volume [V_c] and peripheral volume [V_p]) terms of the semiphysiologic population model. The CL_d and V_p parameters increased with body weight as shown by the regression lines.

Table III. Mean population parameter estimates obtained with the standard two-stage (PCNONLIN) and the nonlinear mixed-effect model (P-Pharm) methods, with use of the semiphysiologic function

	Two-stage method		*NMEM method*	
Sirolimus parameters	*Mean*	*Interpatient variability (CV%)*	*Mean*	*Interpatient variability (CV%)*
CL/F (L/hr)	9.32	47.0	8.91	38.2
V_C (L)	129.9	81.4	112.9	31.8
CL_d (L/hr)	27.5	65.6	25.2	31.9
V_p (L)	462.5	42.4	452.0	26.4
k_a (hr^{-1})	6.50	93.0	2.18	41.3
t_{lag} (hr)	0.29	51.7	0.24	40.1
B/P ratio	34.4	51.2	33.5	48.6

CL/F, Apparent oral clearance; V_C, central volume of distribution; CL_d, distribution clearance; V_p, peripheral volume of distribution.

81.4%), whereas similar values were obtained from the two methods for the peripheral volume.

For the elimination clearance, the NMEM mean and interpatient variability values were 8.91 L/hr and 38.2%, which were lower than the biexponential model estimates of 9.24 L/hr and 53.4%. For the distribution clearance, the NMEM population mean was 25.2 L/hr (CV, 31.9%), which was similar to the two-stage approach, 27.5 L/hr (CV, 65.6%).

For k_a, the P-Pharm population mean (interpatient variability) was 2.18 hr^{-1} (CV, 41.3%), which was very close to that from the biexponential model, 2.77 hr^{-1} (CV, 48.4%). One outlier value with a k_a of 0.29 hr^{-1} was found with P-Pharm. For t_{lag}, both

methods gave similar values, but no outlier was found with NMEM compared with the two-stage method.

The validation of sirolimus population pharmacokinetic estimates obtained by both population approaches with the semiphysiologic model was reflected by the low mean percentage AUC bias ± SD: 5.41% ± 9.95% for the P-Pharm method and –2.15% ± 5.5% for the two-stage method. No individual bias was below –15%, but three were found above 15% with P-Pharm, which may have been attributable to the poor characterization of the distribution phase.

Cyclosporine pharmacokinetics

The mean whole blood cyclosporine concentrations obtained before and after sirolimus oral administration are shown in Fig. 5. Cyclosporine pharmacokinetics was not affected by sirolimus, inasmuch as the noncompartmental estimates of cyclosporine C_{max}, time of observed C_{max}, and $t_{1/2\beta}$ (not included in this report) were not modified. The individual cyclosporine AUC values were similar among centers. The mean ± SD exposure before sirolimus dosing was 4411 ± 2196 ng · hr/ml (range, 808 to 11911 ng · hr/ml); after sirolimus dosing it was 4581 ± 2328 ng · hr/ml (range, 815 to 12956 ng · hr/ml). A paired *t* test showed no significant modification of cyclosporine AUC values before or after sirolimus exposure.

DISCUSSION

The analysis presented in this report provides a comparison of an initial population pharmacokinetic characteristics of sirolimus obtained from two-stage (PCNONLIN) and NMEM (P-Pharm) analyses after the administration of four different oral sirolimus doses (3, 5, 10, and 15 mg/m^2) to kidney transplant patients from three study centers who were receiving concomitant steady-state doses of cyclosporine. To characterize sirolimus whole blood and plasma data, the population analysis was performed with use of a biexponential function with first-order absorption and a t_{lag}. Equations for blood and plasma concentrations as a function of time were written both in terms of intercepts and slopes (biexponential model) and in terms of clearances and volumes (semiphysiologic model). The lack of influence of study site allowed a global analysis of the data.

Properties of sirolimus

With the biexponential model, the intercept coefficients normalized by dose (A and B) were not influenced by doses ranging from 3 to 15 mg/m^2. In addition, the slope (α and β) and absorption parameters (t_{lag} and k_a) were found to be dose-independent. These findings, together with the observed proportionality between sirolimus AUC and dose, suggest that the kinetics of sirolimus was linear over the oral dose range studied. Findings from the semiphysiologic model provided the same conclusion concerning linearity; all clearance and volume parameters were independent of dose.

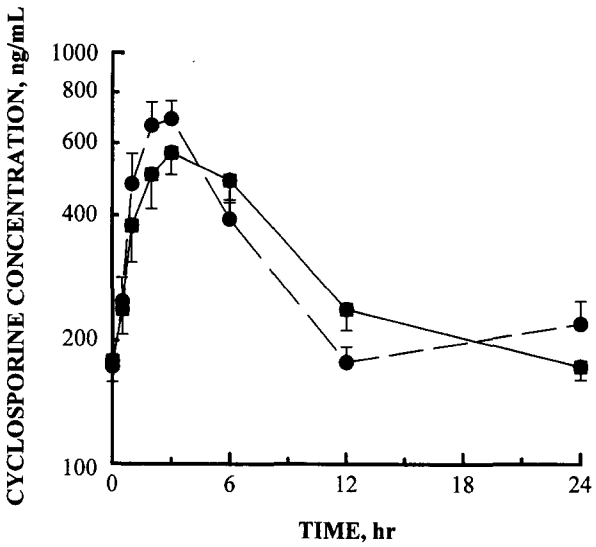

Fig. 5. Cyclosporine concentrations (mean ± SE) as a function of time, before *(squares)* and after *(circles)* sirolimus administration.

Both the biexponential and semiphysiologic population kinetic models showed a correlation of the distribution parameters with body weight and surface area (e.g., peripheral volume of distribution, distribution clearance, and slope; Fig. 4). However, no influence of weight (50 to 100 kg) on the central volume of distribution, elimination clearance, and slope parameters was found (Figs. 3 and 4). The apparent volume of distribution was high, indicating extensive tissue distribution or binding if appreciable absorption of the drug occurs. The influence of the body weight on the distribution parameters may relate to the lipophilicity of sirolimus because this drug has a high partition coefficient, as do tacrolimus and cyclosporine, and may be distributed in part into fat tissues.[10,20]

The whole blood concentrations of sirolimus averaged a factor of 31- to 35-fold higher than

plasma concentrations, showing extensive sirolimus uptake by formed blood elements (Fig. 2). The in vivo blood/plasma ratio of sirolimus was considerably higher than the value of 11 ± 2 obtained by Yatscoff et al.[21] after spiking blood in vitro at concentrations of 5 to 100 ng/ml. The latter study showed no in vivo influence of dose or red blood cell concentrations (0.25 to 230 ng/ml) on sirolimus uptake into the red blood cells. However, these results differ from the nonlinear binding of tacrolimus to FK binding protein (FKBP) and cyclosporine to cyclophylin in the red blood cells.[20,22] Although sirolimus also binds to FK binding protein, the reason for the dissimilar red blood cell binding of sirolimus and tacrolimus is not known. Cyclosporine and sirolimus bind specifically to different red blood cell proteins, explaining the lack of influence of cyclosporine on the blood/plasma ratio of sirolimus.

Sirolimus has been studied in patients with stable kidney transplants who were administered single intravenous doses of 0.5, 1, 2, and 3 mg/m^2 sirolimus (Wyeth-Ayerst Research, unpublished data, 1994). That study yielded a mean $t_{1/2\beta}$ value of 68 hours, which was very close to the P-Pharm population mean value of 63 hours. The mean values were 0.024 L/hr/kg for the intravenous clearance and 1.8 L/kg for the steady-state volume of distribution. With the P-Pharm population analysis, the elimination clearance was 0.127 L/hr/kg and the apparent steady-state volume of distribution was 7.73 L/kg. A comparison between these studies suggests that the systemic oral availability of sirolimus is about 20%. These findings support the characterization of sirolimus as a drug with an apparent low clearance and extensive tissue distribution, thereby showing similarities to tacrolimus[20] and cyclosporine.[10] The noncompartmental analysis of the data from the Swedish patients has also been reported.[11]

Comparison with tacrolimus

The assessment of sirolimus semiphysiologic parameters allows a comparison with tacrolimus pharmacokinetics in a population of liver transplant patients.[20] The steady-state blood volume of distribution of sirolimus (1.756 L/kg) is double that of tacrolimus (0.906 L/kg); the steady-state plasma volume values also differ (58.9 L/kg for sirolimus versus 30.1 L/kg for tacrolimus). Sirolimus blood elimination clearance was one-half tacrolimus clearance (24 versus 54 ml/hr/kg), which was probably due to the lower blood/plasma ratio for tacrolimus (10-30 for tacrolimus versus 33.5 for sirolimus). Sirolimus is more sequestered in the red blood cells, making less drug available for metabolic elimination. Indeed, both compounds undergo an extensive liver metabolism by CYP3A4 enzymes,[22,23] followed by biliary excretion of the metabolites, as does cyclosporine.[24,25] Furthermore, presystemic metabolism by CYP3A4 enzymes in the intestinal mucosa may in part account for the low oral availability of the three immunosuppressants (sirolimus [20%], tacrolimus [25% ± 10%], and cyclosporine [2% to 89%]). Thus the interindividual variability in the systemic oral availability observed with these three immunosuppressants may relate to different intestinal P450 activity and content.[22] However, studies in rabbits suggest that the poor solubility of tacrolimus also results in incomplete absorption.[26] Moreover, the absorption characteristics of sirolimus and tacrolimus are similar. These two drugs show brief t_{lag} values of 0.27 hour (sirolimus) and 0.39 hour (tacrolimus), rapid absorption as reflected by k_a values of 2.77 hr^{-1} and 4.48 hr^{-1}, and large k_a variability of 48% and 67%. However, sirolimus was given in an oral solution, whereas capsules were used for tacrolimus. Thus sirolimus and tacrolimus pharmacokinetics are similar except for the blood/plasma ratio, which is markedly concentration-dependent for tacrolimus. However, further comparisons should be made by use of similar patient types.

Effect on cyclosporine

Cyclosporine blood concentrations were measured before and after sirolimus administration (Fig. 5). No significant modification of cyclosporine oral-dose clearance was observed, because the cyclosporine C_{max} and AUC remained constant. Moreover, cyclosporine exposure was not a significant covariable in explaining the interpatient variability of the sirolimus pharmacokinetic parameters. Therefore coadministration of steady-state doses of cyclosporine and single doses of sirolimus did not produce a pharmacokinetic interaction in the three groups of kidney transplant recipients. It remains to be determined whether these drugs interact in vivo at the level of the immunosuppressive effects in humans; synergistic interactions have been found in vitro in the inhibition of proliferation of normal human periph-

eral blood lymphocytes and in rats with heterotopic cardiac allografts.[27]

In summary, the analysis presented in this report shows that the NMEM method (P-Pharm) provides a better characterization of all sirolimus kinetic phases than does the two-stage method. Individual parameters were defined with more accuracy, and the interpatient variability was estimated more precisely with P-Pharm. In the three patient groups studied, interpatient variabilities of pharmacokinetic parameters were generally less than 40%. Body weight and surface area were the only patient characteristics that showed a significant influence on the pharmacokinetics of sirolimus. Sirolimus exposure (C_{max} and AUC) was proportional to dose over a dose range from 3 to 15 mg/m^2. No pharmacokinetic interactions were observed between sirolimus and cyclosporine.

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References

1. Vezina C, Kudelski A, Sehgal S. Rapamycin (AY-22,989), a new antifungal antibiotic; I: taxonomy of the producing streptomycete and isolation of the active principle. J Antibiot (Tokyo) 1975;28: 721-6.
2. Morris RE. Rapamycins: antifungal, antitumor, antiproliferative, and immunosuppressive macrolides. Transplant Rev 1992;6:39-87.
3. Metclafe SM, Richards FM. Cyclosporine, FK506, and rapamycin: some effects on early activation events in serum-free, mitogen-stimulated mouse spleen cells. Transplantation 1990;49:798-803.
4. Whiting PH, Woo J, Adam BJ, Hasan NU, Thomson AW. The effect of rapamycin on renal function in the rat: a comparative study with cyclosporine. Toxicol Lett 1991;58:169-79.
5. Michnick SW, Rosen MK, Wandless TJ, Karplus M, Schreiber SL. Solution structure of FKBR, a rotamase enzyme and receptor for FK506 and rapamycin. Science 1991;252:836-42.
6. Price DJ, Grove LR, Calvo V, Avruch J, Bierer BE. Rapamycin-induced inhibition of the 70-kilodalton S6 protein kinase. Science 1992;257:973-7.
7. Kay JE, Kromwel L, Doe SEA, Denyer M. Inhibition of T and B lymphocyte proliferation by rapamycin. Immunology 1991;72:544-9.
8. Kahan BD, Gibbons S, Tejpal N, Chou TC, Stepkowski S. Synergistic effect of the rapamycin-cyclosporine combination: median effect analysis of in vitro immune performances by human T lymphocytes in PHA, CD3, and MLR proliferative and cytotoxicity assays. Transplant Proc 1991;23: 1090-1.
9. Zheng B, Shothouse R, Masek MA, Berry G, Billingham ME, Morris RE. Effects of the new and highly active immunosuppressant, rapamycin on lymphoid tissues and cells in vivo. Transplant Proc 1991;23: 851-5.
10. Yee GC. Recent advances in cyclosporine pharmacokinetics. Pharmacotherapy 1991;11:130S-4S.
11. Brattstrom C, Tyden G, Herlenius G, Claesson K, Zimmerman J, Groth CG. Kinetics and dynamics of single oral doses of sirolimus in sixteen renal transplant recipients. Ther Drug Monit 1997 [in press].
12. Peters DH, Fitton A, Ploster GL, Faulds D. Tacrolimus: a review of its pharmacology, and therapeutic potential in hepatic and renal transplantation. Drugs 1993;46:746-94.
13. Takada K, Katayama N, Kiriyama A, Usuda H. Distribution characteristics of immunosuppressants FK506 and cyclosporine in the blood compartment. Biopharm Drug Dispos 1993;14:659-72.
14. Sheiner LB, Raymond JL. Population pharmacokinetic/pharmacodynamic analysis. Drug Inform J 1991; 25:319-21.
15. Gomeni R, Pineau G, Mentre F. Population kinetics and conditional assessment of the optimal dosage regimen using the P-Pharm software package. Anticancer Res 1994;14:2321-6.
16. P-Pharm, a population pharmacokinetic-dynamic data modeling program; user's manual, version 1.3a. Creteil, France: SIMED, Biostatistics and data modeling, 1994.
17. Mentre F, Gomeni R. A two-step iterative algorithm for estimation in nonlinear mixed-effect models with an evaluation in population pharmacokinetics. J Biopharm Stat 1995;5:141-58.
18. Sokal RR, Rohlf FJ. Biometry. San Francisco: WH Freeman, 1961.
19. Bolton SP. Pharmaceutical Statistics. New York: Marcel Dekker, 1986.
20. Jusko WJ, Piekoszewski W, Klintmalm GB, Shaefer MS, Hebert MF, Piergies AA, et al. Pharmacokinetics of tacrolimus in liver transplant patients. Clin Pharmacol Ther 1995;57:281-90.
21. Yatscoff R, LeGatt D, Keenan R, Chackowsky P. Blood distribution of rapamycin. Transplantation 1993;56:12-6.
22. Christians U, Sattler M, Schiebel HM, et al. Isolation of two immunosuppressive metabolites after in vitro metabolism of rapamycin. Drug Metab Dispos 1992; 20:186-191.
23. Sattler M, Guengerich P, Yun CH, Christians U, Sewing KF. Cytochrome P-450 3A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. Drug Metab Dispos 1992;20:753-61.
24. Quesniaux VFJ. Pharmacology of cyclosporine (Sand-

immune); III: immunochemistry and monitoring. Pharmacol Rev 1989;41:249-56.
25. Turgeon DK, Leichtman AB, Lown KS, Normolle DP, Deeb GM, Merion RM, et al. P450 3A activity and cyclosporine dosing in kidney and heart transplant recipients. Clin Pharmacol Ther 1994;56:253-60.
26. Piekoszewski W, Chow FS, Jusko WJ. Disposition of tacrolimus (FK 506) in rabbits, role of red blood cell binding in hepatic clearance. Drug Metab Dispos 1993;21:690-8.
27. Kahan BD, Gibbons S, Tejpal N, Stepkowski SM, Chou TC. Synergistic interactions of cyclosporine and rapamycin to inhibit immune performances of normal human peripheral blood lymphocytes in vitro. Transplantation 1991;51:232-9.