Prediction of Acute Graft Rejection in Renal Transplantation: The Utility of Cyclosporine Blood Concentrations[1]

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While cyclosporine is recommended to be used only in conjunction with monitoring of its blood concentrations, the utility of these measurements in preventing treatment failure is not established. In a group of 52 patients trough levels and steady-state concentrations were monitored in serum and whole blood by specific (SP) and nonspecific (NS) assays (polyclonal radioimmunoassay, PR; fluorescence polarization immunoassay, FP; high-pressure liquid chromatography, HP). From as many as 10 determinations of trough level and steady state concentrations during the first 40 days after renal transplantation, the lowest measurement was selected. In the case of an acute rejection episode within that time period, only values until that event were considered. Trough level measurements in serum by PR/NS and by FP/NS and in whole blood by HP/SP were not significantly different between patients with and patients without rejection episodes. However, simultaneously measured steady-state values (serum/PR/NS and serum/FP/NS) were significantly lower in patients suffering from rejection (with rejection SS/serum/PR/NS mean = 127 ng/ml, SD = 41 ng/ml; without rejection mean = 163 ng/ml, SD = 60 ng/ml; $P = 0.027$, t test). This difference could not be demonstrated for steady state/whole blood/HP/SP measurements. A logistic regression analysis demonstrated that the probability of rejection can be decreased by up to 40% if steady state/serum/PR/NS or steady state/serum/FP/NS values never drop below 250 ng/ml early after renal transplantation.

KEY WORDS: cyclosporin blood concentrations; acute renal graft rejection; therapeutic cyclosporin monitoring; cyclosporin analysis.

INTRODUCTION

A new strategy of optimizing cyclosporine dosage in individual patients used pretransplant pharmacokinetic studies to determine the initial dose rate after renal transplantation, and it uses steady-state concentrations thereafter to adjust these rates if necessary (1). The strategy affords cyclosporine blood concentrations within the targeted range with a higher precision than conventional trough-level monitoring (2). Temptative target ranges are defined for several of the currently available assay methods in serum and whole blood (3).

The present analysis compares the clinical utility of trough level and steady-state concentration monitoring in serum by the nonspecific polyclonal radioimmunoassay and fluorescence polarization immunoassay and in whole blood by specific high-pressure liquid chromatography. Specifically the question is asked whether a low concentration of cyclosporine which persisted for at least 3 days is associated with a higher risk of a rejection episode during the following days.

METHODS

Fifty-two consecutive renal transplant patients were considered in this retrospective analysis between January 1988 and October 1988. The total number of patients transplanted in that time period was 65. Patients receiving prophylactic antilymphocyte globulin on day 1 after transplantation and those experiencing hyperacute rejection between day 1 and day 3 were not included in the analysis. Among the group of 52, 10% received second- or higher-order transplants, 19% were diabetic before transplantation, 35% received grafts from living-related donors, and 36% were female. Among the patients of nonwhite race (42%), Afro-Americans were the largest group (19% of the total population). The mean age was 40 years (median = 40, SD = 12, min = 18, max = 65), and the mean weight was 69 kg (median 66, SD = 18, min = 40, max = 133). Only acute rejection episodes occurring between day 3 and day 40 after transplantation were counted in the analysis. Each episode was confirmed by a biopsy and by a reevaluation of the clinical course 6 months after transplantation.

All patients received individualized intravenous and oral starting doses according to the pharmacokinetic monitoring strategy (1). Analysis of blood samples drawn between 44 and 48 hr after the start of the continuous cyclosporine infusion indicated that steady-state concentrations were attained. For patients undergoing several absorption tests of oral cyclosporine (1), additional infusion steady-state concentrations were available. During the third oral dosing interval without infusion a series of blood samples was obtained (0, 2, 4, 6, 10, 14, and 24 hr for once-daily dosing; 0, 2, 4, 8, and 12 hr for twice-daily dosing), and the area under the drug concentrations–time curve at steady state (AUC_{ss}) was calculated by the trapezoidal rule. An average concentration at steady state was obtained during oral dosing by dividing the AUC_{ss} by the dosing interval. The steady-state concentration data sets which were compared to trough levels were obtained in 25% of the sets during continuous infusions and in 75% during oral dosing. The trough-level data sets consisted of the 12- and 24-hr concentrations of the AUC_{ss} during oral twice-daily and once-daily dosing, respectively. During the intravenous infusion of cyclosporine, the last in each series of steady-state concentrations was

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designated a trough level. This definition of trough levels does not follow pharmacokinetic rules, but it follows the practical use of cyclosporine concentrations by most transplant physicians and is, therefore, appropriate for the present type of comparison.

Blood samples were either collected in tubes containing ethylenediaminetetraacetic acid (EDTA) disodium salt (for whole blood) or clotted in plain tubes at room temperature for more than 2 hr (serum). At each time point, both types of samples were obtained. Serum samples were analyzed by two types of nonspecific assay methods: a polyclonal radioimmunoassay of Sandoz (4) and a polyclonal fluorescence polarization immunoassay of Abbott (5). The content of unchanged cyclosporine was specifically determined in whole blood samples by a high-pressure liquid chromatographic assay using solid phase extraction as sample preparation (6).

Standard hypothesis testing was used for the initial comparison between data sets. The distribution of each data set was tested for normality by the Wilk–Shapiro test. Normally distributed sets were compared by the t test; all other sets were compared by the signed-rank test. When blood concentrations of rejecting and nonrejecting patients showed a difference of at least borderline significance, two types of regression analyses of the probability of rejection versus the concentration were performed. First, a nonparametric regression line was drawn for the declining probability of rejection with increasing blood concentrations. This line was smoothed by the LOWESS technique (7). Second, the data was fitted by a logistic regression model (SAS statistical software):

$$p(\text{rj}) = \frac{s}{n} = \frac{e^u}{1 + e^u}$$

where p(rj) is the probability of rejection, s is the number of patients with rejection, n is the total number of patients, and u is the linear function of the independent variable, i.e., the concentration u = intercept + slope · concentration.

The two regression parameters (intercept and slope) were estimated by a maximum-likelihood method. The goodness of fit of the model was evaluated by the chi-square statistics.

RESULTS

The cyclosporin concentrations (Table I) reflect either an intravenous or an oral steady-state dosing situation. The lowest trough-level or steady-state concentration before or at a rejection episode or during the first 40 days posttransplant in the absence of such episodes is listed. There was no significant difference between trough levels in patients with and patients without rejection episodes. This finding was consistent in all three assays. Also, steady-state concentrations measured by the specific liquid chromatographic method in whole blood did not differ between the two groups of patients. The difference between serum steady-state concentrations in rejecting and nonrejecting patients as measured by the fluorescence polarization immunoassay reached borderline significance. A much clearer distinction was reached by assaying the same samples with the radioimmunoassay. The lowest steady-state concentration experienced by patients with rejection (mean = 127 ng/ml, SD = 41 ng/ml) was significantly different (P = 0.027) from the lowest concentration in patients without rejection (Table I).

Those two data sets consisting of steady-state concentrations and displaying borderline or full significance were subjected to two types of regression analyses displayed in Figs. 1 and 2. The nonparametric regression provided a visual impression of the decline in the probability of rejection with increasing concentrations. A logistic regression analysis provided a continuous model for the empiric relationship. The regression parameters and goodness-of-fit statistics are listed in Table II. The shallower slope of the regression for the fluorescence polarization immunoassay (−0.0093 ml/ng) compared to the radioimmunoassay (−0.014 ml/ng) confirmed the impression given by the nonparametric regression. The logistic regression models predicted that the probability of acute rejection could be reduced up to 40% if steady-state concentrations never fell below 250 ng/ml in serum as determined by the polyclonal assays.

DISCUSSION

Even though blood samples for area-under-the-curve monitoring were obtained from one defined group of 52 renal transplant patients, the unintended loss of samples resulted

Table I. Cyclosporine Blood Concentrations in Patients With and Without Acute Graft Rejection

	TL/SE/PR/NS[a]		SS/SE/PR/NS		TL/SE/FP/NS		SS/SE/FP/NS		TL/WB/HP/SP		SS/WB/HP/SP	
Rejection	N	Y	N	Y	N	Y	N	Y	N	Y	N	Y
Mean[b]	—	84	163	127	—	—	156	120	—	—	268	242
Median[b]	68	—	—	—	68	65	—	—	104	99	—	—
SD	—	47	60	41	—	—	75	48	—	—	108	102
Range	19–187	—	—	—	23–244	15–165	—	—	41–467	45–301	—	—
n	33	19	33	19	32	19	32	19	29	16	29	16
SL[c]	P = 0.66		P = 0.027		P = 0.86		P = 0.066		P = 0.78		P = 0.44	

[a] TL, trough level; SS, steady-state concentration; SE, serum; WB, whole blood; PR, polyclonal radioimmunoassay; FP, fluorescence polarization immunoassay; HP, high-pressure liquid chromatography; NS, nonspecific; SP, specific for cyclosporine.

[b] For normally distributed data (Wilk–Shapiro test), the mean and standard deviation are reported. For log-normally distributed data, the median and range are reported.

[c] Significance level of the statistical test. When all data were normally distributed, the t test was used; otherwise, the signed-rank test was employed.

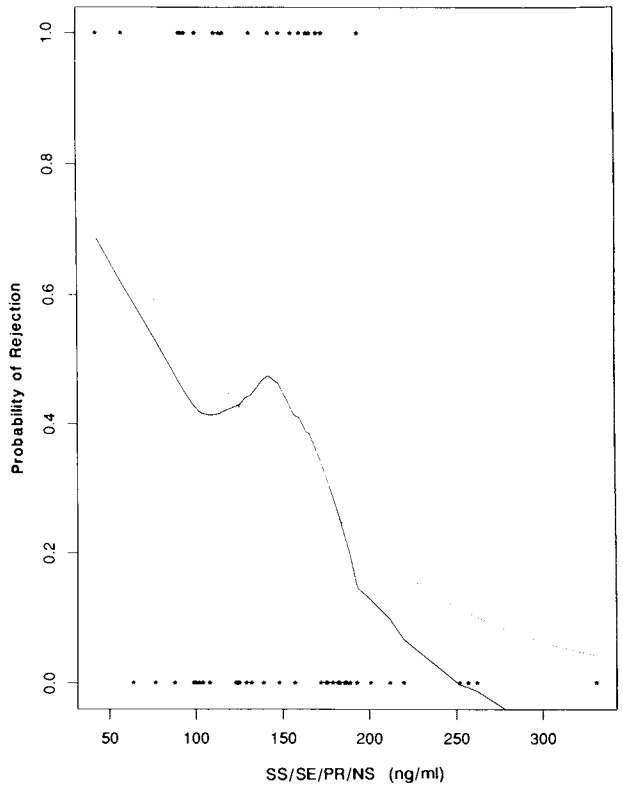

Fig. 1. Regression analysis of the probability of rejection (ordinate) versus the lowest steady-state concentration measured in serum by a nonspecific polyclonal radioimmunoassay before or at the time of the rejection or during the first 40 days after transplantation in the absence of acute rejections (abscissa). The stars indicate the lowest concentration experienced by patients without rejection (at bottom; n = 33) and by patients with rejection (at top; n = 19). The solid line is the result of a nonparametric regression analysis, and the dotted line represents the best fit to a logistic regression model.

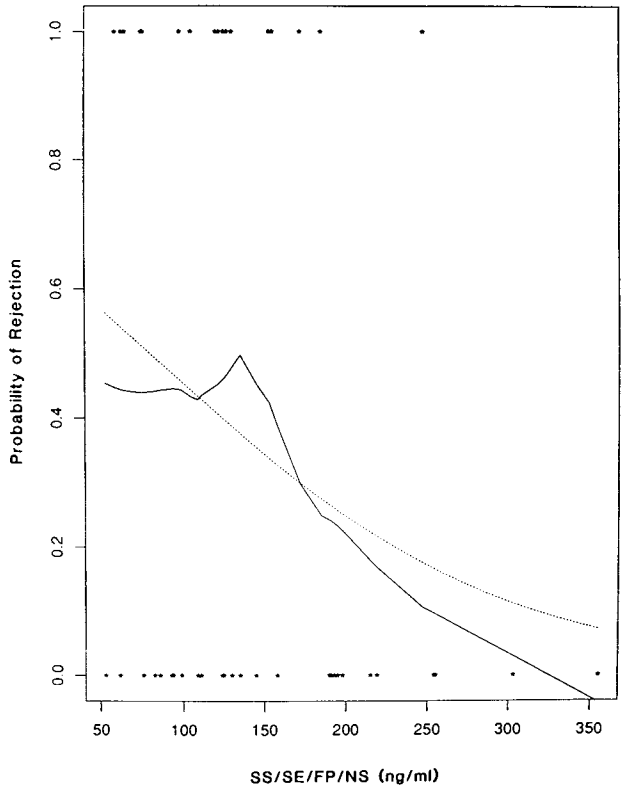

Fig. 2. Regression analysis of the probability of rejection (ordinate) versus the lowest steady-state concentration measured in serum by a nonspecific fluorescence polarization immunoassay before or at the time of the rejection or during the first 40 days after transplantation in the absence of acute rejections (abscissa). The stars indicate the lowest concentration experienced by patients without rejection (at bottom; n = 32) and by patients with rejection (at top; n = 19). The solid line is the result of a nonparametric regression analysis, and the dotted line represents the best fit to a logistic regression model.

in a different total number of patients in the three assay groups: n = 52 for serum radioimmunoassay, n = 51 for serum fluorescence polarization immunoassay, and n = 45 for whole-blood liquid chromatography. There is no reason to expect that these different numbers should have influenced the overall comparison.

According to the monitoring strategy (1), a minimum of five determinations of steady-state concentrations and trough levels was available for each patient during the first 40 days posttransplant, unless graft loss caused a premature end to cyclosporine immunosuppression. More frequent monitoring was performed in patients requiring adjustments of the oral dose rate. In agreement with a previous study (8), trough levels measured at the time of rejection were not significantly different from trough levels measured in the absence of rejection (data not shown). The same was true for steady-state concentrations at the time of rejection. Furthermore, also concentrations obtained during 7 days preceding the rejection episode were not significantly lower than control concentrations not preceding rejection (data not shown). Furthermore, a low cyclosporine concentration may allow the maturation of competent cytotoxic T cells whose subsequent clonal amplification cannot be stopped by cyclosporine and may lead to a rejection episode days later. The lowest steady-state concentration preceded the rejection episode by up to 48 hr in 11 of 19 cases. In the remaining eight cases the lowest concentration was measured between 3 and 8 days before the episode. These findings were the basis for selecting the lowest concentration before a rejection event as the discerning variable in the investigation of the utility of different cyclosporine concentrations. This selection was the result of an empiric process, and the present analysis can, therefore, not exclude certain clinical utilities for any of the cyclosporine concentrations. Most noticeably, cyclosporine-induced toxicities are not part of this analysis, mainly because the frequency was very low (e.g., 10% for nephrotoxicity and 0% for hepatotoxicity).

The superiority of steady-state concentrations over trough levels can be explained when one considers that during the intravenous infusion as well as during oral dosing, steady-state concentrations are derived from at least five individual concentrations, and they reflect, therefore, the overall exposure of a patient to cyclosporine much better than a single determination such as a trough level. On the other hand, trough levels also were obtained only during steady-state dosing (infusion for 2 days or oral dosing for three intervals without a change in the dose rate) and are

Table II. Parameters of the Logistic Regression of the Probability of Rejection Versus Steady-State Concentration

Concentration[a]	Slope[b] (ml/ng)	Intercept[b]	Significance[c]
SS/SE/PR/NS	−0.014 (0.0065)	1.43 (0.95)	−2 log-likelihood ratio = 5.44 $P = 0.02(\chi^2\ 1\ df)$
SS/SE/FP/NS	−0.0093 (0.0052)	0.75 (0.74)	−2 log-likelihood ratio = 3.74 $P = 0.05(\chi^2\ 1\ df)$

[a] SS, steady-state concentration; SE, serum; PR, polyclonal radioimmunoassay; FP, fluorescence polarization immunoassay; NS, nonspecific.

[b] Parameters of linear function u of logistic model $e^u/(1 + e^u)$.

[c] The two-parameter logistic model offered a significant improvement over a one-parameter model.

representing more than just a single moment of cyclosporine activity preceding a rejection episode.

Among the data sets of steady-state concentrations, the difference between the specific measurement (liquid chromatography) in whole blood and the nonspecific measurement in serum was unexpected. At the present time, no data are available to differentiate between two possible explanations: (i) about half of the cyclosporine in whole blood is associated with red blood cells (9) and is, therefore, not available for immunosuppressive effects; and (ii) metabolites contribute to the therapeutic effect of cyclosporine (10), and a nonspecific assay, therefore, affords a more relevant measurement. Both arguments, however, can be countered: (i) cyclosporine bound to lipoproteins can interact with hepatocytes (11), and cyclosporine bound to red blood cells may interact with lymphocytes; and (ii) a recent conference on cyclosporine monitoring provided no convincing evidence for a therapeutic role of cyclosporine metabolities (12).

The good agreement between nonspecific measurements of steady-state concentrations in serum confirms the close correlation between the radioimmunoassay and the fluorescence polarization immunoassay which was recently published (3). The slope for the regression line in the relationship with the probability of rejection is, however, shallower in the case of the fluorescence polarization assay. This assay, consequently, does not predict the probability of rejection as reliably as the radioimmunoassay. The difference may be related to the use of different polyclonal antisera in the two assays.

In conclusion, steady-state concentration monitoring which includes the measurement of areas under the curve after oral dosing affords a prediction of the probability of rejection, which is not reached by conventional trough-level monitoring. At the present time, it seems as if this benefit can be gained only by nonspecific measurements of cyclosporine in serum. Additional studies are necessary to define the role of specific measurements in whole blood.

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REFERENCES

1. B. D. Kahan and J. Grevel. Optimization of cyclosporine therapy in renal transplantation by a pharmacokinetic strategy. *Transplantation* 46:631–644 (1988).
2. J. Grevel, M. S. Welsh, and B. D. Kahan. Cyclosporine monitoring in renal transplantation: Area under the curve monitoring is superior to trough-level monitoring. *Ther. Drug Monit.* 11:246–248 (1989).
3. J. Grevel, K. L. Napoli, S. Gibbons, and B. D. Kahan. Area-under-the-curve monitoring of cyclosporine therapy: Performance of different assay methods and their target concentrations. *Ther. Drug Monit.* 12:8–15 (1990).
4. P. Donatsch, E. Abisch, M. Homberger, R. Traber, M. Trapp, and R. Voges. A radioimmunoassay to measure cyclosporin A in plasma and serum samples. *J. Immunoassay* 2:19–32 (1981).
5. P. Wang, M. A. Morrison, and N. Wang. A fluorescence polarization immunoassay (FPIA) for the quantitation of cyclosporin. *Clin. Chem.* 32:1061–1062 (1986) (abstr.).
6. Bio-Rad. *Bio-Rad Cyclosporine by HPLC Test, Instruction Manual,* Catalog No. 195-7022, Bio-Rad Laboratories, Clinical Division, Hercules, CA, Sept. 1986.
7. J. M. Chambers, W. S. Cleaveland, B. Kleiner, and P. A. Tukey. *Graphical Methods for Data Analysis,* Wadsworth International Group and Duxbury Press, Belmont, CA, 1983.
8. D. W. Holt, J. T. Marsden, A. Johnston, M. Bewick, and D. H. Taube. Blood cyclosporine concentrations and renal allograft dysfunction. *Br. Med. J.* 293:1057–1059 (1986).
9. R. P. Agarwal, R. A. McPherson, and G. A. Threatte. Assessment of cyclosporin A in whole blood and plasma in five patients with different hematocrit. *Ther. Drug. Monit.* 7:61–65 (1985).
10. T. G. Rosano, B. M. Freed, J. Cerilli, and N. Lempert. Immunosuppressive metabolities of cyclosporine in the blood of renal allograft recipients. *Transplantation* 42:262–267 (1986).
11. S. K. Gupta and L. Z. Benet. High-fat meals increase the clearance of cyclosporine. *Pharm. Res.* 7:46–48 (1990).
12. B. D. Kahan, L. M. Shaw, D. Holt, J. Grevel, and A. Johnston. Consensus document: Hawk's lay meeting on therapeutic drug monitoring of cyclosporine. *Clin. Chem.* 36:1510–1516 (1990).