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ORIGINAL ARTICLE

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Time-dependent changes in cyclosporine exposure: implications for achieving target concentrations

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Abstract This study analyzed early changes in trough blood cyclosporine concentrations and cyclosporine exposures after kidney transplantation. Seventy-two patients who received cyclosporine-based immunosuppressive therapy were intensively monitored (C0) during the first 6 months after transplantation. Full pharmacokinetic studies were performed at day 4, and months 2, 3, and 6 after transplantation. Mean steady-state, dose-adjusted trough cyclosporine blood concentrations increased from 1.1 ± 0.60 (day 7) to 2.0 ± 1.20 ng/ml per mg (day 30, $P < 0.01$). Steady-state, dose-adjusted cyclosporine exposure parameters (C0, Cmax, AUC, Cavg, and C12) were significantly lower at day 4 than at months 2, 3, and 6 after transplantation ($P < 0.01$). Initial cyclosporine doses produced target concentrations in only 30% of the patients at day 3. C2 was the single concentration that showed high and consistent correlation with serial AUC measurements ($r^2 \geq 0.76$). The incidence of biopsy-proven acute rejection was 20.5% and was not associated with ethnicity, HLA mismatch, adjunctive therapy, or blood trough cyclosporine concentrations below 200 ng/ml at day 3. Significant time-dependent increases in steady-state cyclosporine exposure occur during the first month after kidney transplantation. Due to the low relative bioavailability early after surgery, higher doses and more frequent cyclosporine dose adjustments are necessary to produce target exposures early after transplantation.

Keywords Kidney transplantation · Cyclosporine · Pharmacokinetics · Therapeutic drug monitoring

Introduction

The wide clinical use of the microemulsion formulation of cyclosporine has definitely confirmed its superior pharmacokinetics over the oil-based formulation [1, 25]. Studies performed early after transplantation and also in stable patients, in different types of organ transplantation, have consistently shown improved dose–concentration linearity, reduced between-subject and within-subject variability, stronger concentration–AUC correlations and an improved efficacy/toxicity/tolerability profile [3, 14, 17, 21, 22, 23, 37, 38, 43]. Compared with the oil-based formulation, the higher Cmax and AUC achieved with the microemulsion formulation [7] provide higher exposure, which has been associated with reduced, but still significant, acute rejection rates of 35% in patients receiving cyclosporine, azathioprine, and prednisone [21, 41].

Concurrently, many studies have been performed to perfect therapeutic cyclosporine monitoring after transplantation [7, 9, 10, 35, 40]. The improved pharmacokinetic characteristics of the microemulsion formulation

allow determination of exposure that uses fewer and early blood concentrations [4, 31]. In fact, the monitoring of the AUC from 0–4 h after dosing or even the concentration obtained 2 h after dosing has been correlated with very low incidences of acute rejection [4, 34].

Recently, attention has been paid to the very early post-transplant period, i.e., the first week after transplantation. During this period, physiological and biochemical changes are greater and complicated by anesthesia and postoperative ileus, all significantly affecting the absorption of orally delivered drugs [12] and increasing the risk of acute rejection due to insufficient drug exposure [12]. Although therapeutic cyclosporine concentrations can be achieved more rapidly and with lower doses of cyclosporine microemulsion than with the oil-based formulation [1], the higher initial cyclosporine doses necessary during this period complicate cyclosporine dose adjustment, because these doses lie outside the linear dose–concentration range [26, 38]. Alternatively, pre-transplant cyclosporine test doses, which could minimize this difficulty, have failed to predict consistently and accurately initial doses in the majority of patients and are very difficult to determine, especially in recipients of cadaveric allografts [7]. The importance of finding initial doses of cyclosporine able rapidly to produce target concentrations has recently been underscored in studies in which early obtainment of target drug exposures, between days 3 and 5, were associated with a very low incidence of acute rejection [33, 34].

This study addresses the capacity of routine initial cyclosporine doses to produce therapeutic cyclosporine concentrations during the first week. Moreover, time-dependent changes and variability in cyclosporine exposures were also analyzed during periods of unstable and stable cyclosporine pharmacokinetics so that parameters could be identified that would accurately predict patients at higher risk of developing acute and chronic rejection [19]. Correlations between acute rejection and cyclosporine concentrations obtained early after transplantation or at the time of rejection were also analyzed.

Patients and methods

Patients and immunosuppression

Between 31 March 1999 and 25 September 2000, 72 patients were enrolled in three multicenter and randomized clinical trials to receive cyclosporine-based immunosuppressive therapy after being informed of the nature and details of the study and having signed a written informed consent. The local medical ethics committee approved all the protocols, and the studies were performed in accordance with the Declaration of Helsinki and US Food and Drug Administration guidelines for good clinical practice. All patients received initial cyclosporine doses of 4–5 mg/kg per dose, administered twice daily, beginning 12–24 h after graft re-vascularization. Cyclosporine doses were adjusted to achieve trough blood levels of 200–400 ng/ml during the first 30 days, 150–300 ng/ml between 31 and 90 days, and 100–200 ng/ml thereafter. Adjunctive therapy consisted of fixed doses of azathioprine (AZA, $n=19$), mycophenolate mofetil (MMF, $n=15$), FTY720 ($n=18$), or everolimus (EVL, $n=20$). Large international studies have shown that none of these new drugs significantly affects cyclosporine pharmacokinetics [6, 27]. All patients received 1 g bolus of methylprednisolone administered during transplant surgery. From the second day on, patients received 0.5 mg/kg per day prednisone for 4 weeks. Prednisone doses were tapered to 0.4 mg/kg per day between 29 and 45 days, 0.3 mg/kg per day between 46 and 90 days, and 0.2 mg/kg per day thereafter. All drug doses were also adjusted for safety and tolerability. All suspected acute rejection episodes were confirmed by core needle biopsy and graded according to the 1997 Banff criteria.

Pharmacokinetic studies

All patients signed an additional written informed consent to undergo pharmacokinetic studies. Cyclosporine pharmacokinetics were assessed in fasted patients (10 h) after they had received their previous evening dose 12 h before starting the study. Doses of cyclosporine were administered with milk with the assistance of a pharmacist. Current doses of adjunctive immunosuppressive therapy and other co-medication were then administered. None of the patients was taking known drugs that significantly interfered with cyclosporine pharmacokinetics. Venous blood samples (3 ml) were collected into EDTA-containing tubes that were gently inverted several times and stored at −20 °C till determination of cyclosporine concentrations. To determine early pharmacokinetic variability after transplantation, we obtained time–concentration curves 4 days after transplantation for 14 patients receiving AZA and steroids. Blood samples were collected just before (time 0), and 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after cyclosporine administration. For the between-subject and within-subject pharmacokinetic variability in cyclosporine exposure at steady-state to be determined, 27 patients receiving EVL ($n=18$) or MMF ($n=9$) underwent serial studies at months 2, 3, and 6. Blood samples were collected just before (time 0) and 1, 2, 5, 8, and 12 h after the morning administration of cyclosporine.

Pharmacokinetic analysis

Whole-blood cyclosporine concentrations were analyzed by standard non-compartmental methods using the WinNonlin software (Scientific Consulting). The highest peak concentration and the corresponding sampling time were defined as Cmax and tmax, respectively. C0 and C12 were the concentrations measured just before and 12 h after cyclosporine administration. The AUC was calculated from 0 to 12 h by the linear trapezoidal rule. The average steady-state concentration (Cavg) was calculated as AUC/τ, where τ is the dosing interval. Percent peak trough fluctuation (%PTF) was calculated as $100\times(Cmax-C0)/Cavg$. Between-subject and within-subject variabilities were calculated as the percent coefficient of variation ($CV=SD\times100/mean$) of the mean dose-adjusted cyclosporine pharmacokinetic parameters.

Therapeutic drug monitoring

In all patients trough blood cyclosporine concentrations were measured twice a week during the first 4 weeks, once a week between weeks 4 and 8, every other week between weeks 9 and 12, and every 4 weeks thereafter. Trough blood cyclosporine concentrations of 65 of the 72 patients (90%) who reached 24 weeks of

follow-up were used for analysis. Between-subject variability in dose-adjusted trough blood cyclosporine concentrations was assessed as percent coefficient of variation (%CV) at days 3, 7, 14, 30, 60, 90, and 180 after transplantation. In 23 of the 72 patients (32%) cyclosporine trough concentrations were obtained daily during the first week after transplantation, just before the morning dose of cyclosporine. In these patients, cyclosporine doses were adjusted daily to reach the target therapeutic concentration of 200 ng/ml. Between-subject variability (%CV) in dose-adjusted blood cyclosporine concentrations was also assessed at days 2, 3, 4, 5, 6, and 7 after transplantation. Within-subject variability in dose-adjusted trough blood cyclosporine concentrations was assessed as %CV during three periods after transplantation: between days 2 and 7, days 8 and 30, and days 60 and 180 after transplantation.

Concentration-efficacy correlations

Of the 72 patients, 68 (94%) were eligible for analysis of acute rejection incidence. Four patients were excluded because of early graft thrombosis ($n=3$) or change in the original immunosuppressive protocol. Retrospective analysis of our own data demonstrated that patients with trough cyclosporine concentrations below 200 ng/ml during the first week after transplantation are at higher risk of developing acute allograft rejection. The relationship between ethnicity, HLA mismatch, adjunctive therapy, and trough blood cyclosporine concentration and acute rejection was calculated by logistic regression analysis. The overall incidence of acute rejection during the first 6 months after transplantation was compared between patients who did or did not reach therapeutic trough cyclosporine concentrations (200 ng/ml) between days 3 and 5. Also, acute rejection episodes were compared according to the last trough blood cyclosporine concentrations before the diagnosis of acute rejection.

Bio-analytical method

Whole-blood cyclosporine concentrations were determined by means of the AxSYM cyclosporine fluorescence polarization immunoassay kit (Abbott Laboratories, Ill. USA), according to the manufacturer's directions. Performance was assessed on the basis of a 3-point quality-control concentration range of low (70 ng/ml), intermediate (300 ng/ml), and high (600 ng/ml) concentrations in 32 assays performed on different days. The coefficient of variation was 8, 6, and 7%, and bias was −8.9, −8.7, and −8.7%, respectively. Quantification of the assay limit was 25 ng/ml.

Statistical analysis

Continuous variables were analyzed by paired or unpaired Student's t-tests or by analysis of variance and expressed as mean and standard deviation. We used Tukey's post-hoc range test to identify differences between means. Categorical variables were analyzed by χ^2-tests and expressed as frequencies or median and range. Summary statistics were expressed for observed and dose-adjusted cyclosporine pharmacokinetic parameters stratified by period of observation. We used linear and multiple stepwise linear regressions to calculate correlations between single or multiple cyclosporine concentrations and total AUC. The general linear model for repeated measures (two-way ANOVA) was used for the comparison of pharmacokinetic parameters and dose-adjusted cyclosporine blood concentrations, with subject and visit, or adjunctive drug treatment and visit, used as sources of variation after transplantation, respectively. We used whisker-and-box plots and the Wilcoxon signed rank test to demonstrate and compare within-subject variability. Logistic regression analysis was used for the correlation of ethnicity, HLA mismatch, adjunctive immunosuppression, and blood cyclosporine concentration at day 3 with the incidence of acute rejection, and odds ratios (ORs) are presented. SPSS 7.5 software (SPSS) was used for the statistical analysis. Differences were considered significant at $P<0.05$.

Results

Demographic characteristics

The demographic characteristics of this patient population, as shown in Table 1, are representative of our

Table 1 Demographic characteristics stratified by adjunctive immunosuppressive therapy

Parameter	AZA (19)	FTY720 (18)	MMF (15)	EVL (20)	TOTAL (72)
Age (years)	35.2 ± 8.4	40.6 ± 11.3	40.6 ± 9.8	37.9 ± 13.0	38.4 ± 10.8
Gender (male/female)	9/10	10/8	8/7	10/10	37/35
Ethnicity (white/non-white)	10/9	15/3	9/6	12/8	46/26
Body mass index (kg/m^2)	23.9 ± 4.8	23.0 ± 2.7	23.7 ± 2.9	23.1 ± 3.5	23.3 ± 3.6
End-stage renal disease					
Glomerulonephritis	4	4	4	4	16
Hypertension	7	2	3	3	15
Diabetes	2	3	1	2	8
Other	6	9	7	11	33
Donor source (living/cadaver)	18/1	18/0	14/1	18/2	68/4
HLA mismatches	2.2 ± 0.5	2.9 ± 0.5	3.2 ± 1.4	3.9 ± 1.8[a]	3.0 ± 1.3
PRA (%)	0.0 ± 0.0	0.3 ± 1.0	0.8 ± 1.4	1.0 ± 2.2	0.5 ± 1.4
Dialysis (hemo/peritoneal)	18/1	16/2	14/1	17/3	65/7
Time on dialysis (months)	18.1 ± 10.9	19.1 ± 10.6	16.3 ± 10.6	24.8 ± 24.1	19.8 ± 15.7
Hepatitis C (IgG-positive)	3	0	0	0	3
Hepatitis B (IgG-positive)	0	0	0	0	0
CMV (IgG-positive)	16	9	9	17[b]	51

[a] $P<0.05$, AZA < FTY720 < MMF = EVL
[b] $P<0.05$, AZA = EVL > FTY720 = MMF

general adult transplant population. The mean age was 38.4 ± 10.8 years, and mean body mass index was 23.3 ± 3.6 kg/m^2. Fifty-three percent of the patients were male and 65% were white. The most frequent causes of end-stage renal disease were chronic glomerulonephritis (22%) and hypertension (21%), and these patients were predominantly on hemodialysis (90%) for a mean time of 19.8 ± 15.7 months. The vast majority of the patients were recipients of living related-donor kidney allografts (94%) with mean HLA mismatches and PRA of 3.0 ± 1.3 and 0.5 ± 1.4, respectively. Prevalence of hepatitis C and CMV seropositivity were 4% and 71%, respectively. No significant differences were observed when baseline demographic characteristics were compared, except for mean HLA mismatches and previous CMV infection when patients were stratified by the type of adjunctive immunosuppressive therapy (Table 1).

Pharmacokinetic analysis

Steady-state mean pharmacokinetic parameters are shown in Table 2. Four days after transplantation, peak blood cyclosporine concentrations of 1,269 ± 524 ng/ml were reached 2.1 ± 0.8 h after cyclosporine administration. Mean trough cyclosporine concentrations were not different when C0 and C12 were compared (225 ± 150 vs 170 ± 99 ng/ml, $P = 0.096$; 0.8 ± 0.5 vs 0.6 ± 0.3 ng/ml per mg, $P = 0.067$, respectively; Table 2). The mean cyclosporine AUC was 5,839 ± 2,319 ngxh/ml, and mean PTF was 222 ± 54%. Compared with that at months 2 and 3, steady-state cyclosporine exposure (C0, C12, Cmax, AUC, or Cavg) was significantly lower 6 months after transplantation ($P < 0.01$) simply due to a significant cyclosporine dose reduction, since dose-adjusted cyclosporine exposure parameters were not significantly different. Although studies were not performed on the same transplant population, it is apparent that dose-adjusted cyclosporine exposure (C0, Cmax, AUC, Cavg, and C12) was significantly lower early after transplantation (day 4) than after 2, 3, and 6 months ($P < 0.01$, one-way ANOVA). Between-subject variability in cyclosporine exposure parameters ranged from 25% to 67% and did not change after transplantation ($P > 0.05$, day 4 vs months 2, 3, or 6, unpaired Student's t-test; $P > 0.05$, months 2 vs 3 vs 6, ANOVA). Variability was consistently higher for C0 or C12 than for AUC, Cmax, or Cavg at any time after transplantation (Table 2).

Correlations between concentrations and AUC

Correlations between single blood cyclosporine concentrations and AUC ranged from $r^2 = 0.12$ for C1 at day 4 to 0.88 for C3 at day 4 (Table 3). Among concentrations measured within all pharmacokinetic studies, C2 showed the highest correlation with AUC. C12 tended to show better correlation with AUC than C0 did. The correlations between observed Cmax and AUC were $r^2 = 0.80$, 0.73, 0.65, and 0.88 at day 4 and months 2, 3, and 6, respectively. Using stepwise multiple linear regression analysis, we identified abbreviated concentration-time profiles, using 2, 3, or 4 cyclosporine concentrations. We obtained correlation coefficients (adjusted r^2) of 0.92 or 0.95, using only two or three cyclosporine concentrations. Even when we used only early cyclosporine concentrations, correlation coefficients of 0.95 or higher were observed (Table 3).

Table 2 Cyclosporine pharmacokinetic parameters after transplantation. Values are median [range] for tmax and mean ± standard deviation for all other parameters; %CV in parenthesis

Parameter *n*	Day 4 14	Month 2 27	Month 3 27	Month 6 27
Dose (mg/dose)	282 ± 70 (24)	150 ± 47 (31)	135 ± 42 (31)	99 ± 31 (31)[a]
C0 (ng/ml)	225 ± 150 (67)	180 ± 65 (36)	180 ± 97 (54)	111 ± 55 (49)[a]
Cmax (ng/ml)	1,269 ± 524 (41)	1,464 ± 572 (39)	1,246 ± 468 (38)	1,029 ± 403 (39)[a]
C12 (ng/ml)	170 ± 99 (58)	186 ± 69 (37)	176 ± 98 (56)	108 ± 68 (62)[a]
AUC (ngxh/ml)	5,839 ± 2,319 (40)	6,441 ± 2,047 (32)	5,769 ± 2,212 (38)	3,884 ± 1,434 (37)[a]
Cavg (ng/ml)	487 ± 193 (40)	537 ± 171 (32)	481 ± 184 (38)	323 ± 120 (37)[a]
C0/dose (ng/ml)/mg	0.8 ± 0.5 (59)[c]	1.3 ± 0.6 (44)	1.4 ± 0.7 (47)	1.1 ± 0.4 (39)
Cmax/dose (ng/ml)/mg	4.4 ± 1.1 (25)[c]	10.3 ± 3.8 (38)	9.6 ± 3.5 (37)	10.5 ± 3.0 (29)
C12/dose (ng/ml)/mg	0.6 ± 0.3 (48)[c]	1.3 ± 0.4 (31)	1.3 ± 0.5 (41)	1.1 ± 0.6 (54)
AUC/dose (ngxh/ml)/mg	20.3 ± 5.6 (28)[c]	45.2 ± 13.5 (30)	44.0 ± 13.8 (31)	39.8 ± 10.9 (27)
Cavg/dose (ng/ml)/mg	1.7 ± 0.5 (28)[c]	3.8 ± 1.1 (30)	3.7 ± 1.2 (31)	3.3 ± 0.9 (27)
tmax (h)	2 [1–4] (35)	2 [1–2] (29)	2 [1–5] (51)	1[1–2] (36)
PTF (%)	222 ± 54 (24)	235 ± 62 (26)	224 ± 61 (27)	285 ± 48 (17)[b]

[a] $P < 0.001$, two-way ANOVA, months 2 = 3 > 6
[b] $P < 0.001$, two-way ANOVA, months 2 = 3 < 6
[c] $P < 0.01$, one-way ANOVA, day 4 < months 2 = 3 = 6

Table 3 Coefficients of correlation (adjusted r^2) between cyclosporine blood concentrations and observed AUCs after transplantation. Equations were derived by simple or multiple stepwise linear regression analysis

Concentrations	Day 4	Month 2	Month 3	Month 6
Single				
C0	0.51	0.24	0.46	0.66
C1	0.12	0.44	0.27	0.32
C1.5	0.15			
C2	0.76	0.79	0.81	0.77
C2.5	0.79			
C3	0.88			
C4	0.68			
C5		0.60	0.64	0.72
C6	0.82			
C8	0.64	0.46	0.52	0.66
C12	0.81	0.41	0.72	0.55
Multiple				
Two time points	0.96 (C3, C12)	0.94(C2, C8)	0.92(C2, C5)	0.95 (C2, C1)
Three time points	0.97 (C3, C12, C2)	0.98 (C2, C8, C1)	0.96 (C2, C5, C8)	0.98 (C2, C1, C5)
Four time points	0.99 (C3, C12, C2, C4)	0.998 (C2, C8, C1, C5)	0.997 (C2, C5, C8, C1)	0.995 (C2, C1, C5, C8)
Early time points	0.96 (C3, C1.5, C0)	0.99 (C2, C5, C1)	0.95 (C2, C5, C1)	0.98 (C2, C1, C5)

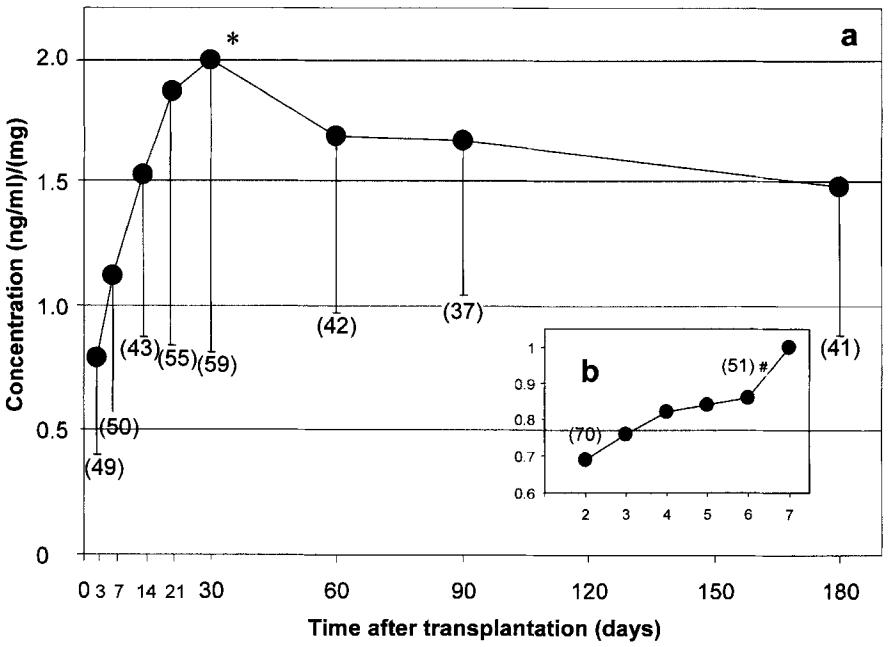

Fig. 1 **a** Mean dose-adjusted cyclosporine blood concentrations between 3 and 180 days after transplantation ($n=65$); $^*P<0.0001$, two-way ANOVA for repeated measures comparing days 3, 7, 14, 21, and 30. **b** Corresponding values obtained between 2 and 7 days after transplantation ($n=23$); $^{\#}P=0.036$, two-way ANOVA for repeated measures comparing days 2, 3, 4, 5, 6, and 7. *Numbers in parenthesis* are percent coefficients of variation (%CV) representing between-subject variability

Therapeutic drug monitoring

The initial cyclosporine dose was 497 ± 160 mg/day. Mean dose-adjusted blood cyclosporine concentrations increased from 0.8 ± 0.4 at day 3 to 1.1 ± 0.6 at day 7 and up to 2.0 ± 1.2 ng/ml per mg at day 30 ($P<0.001$), slowly decreasing to 1.7 ± 0.6, 1.7 ± 0.5, and 1.5 ± 0.6 ng/ml per mg at days 60, 90, and 180, respectively (Fig. 1a). At day 3, patients were receiving 527 ± 108 mg/day cyclosporine, and only 37% showed trough blood cyclosporine concentrations equal to or higher than 200 ng/ml. Adjustment of blood concentrations obtained at day 3 to the previous cyclosporine dose (mg/kg) resulted in a wide range of concentrations, from 14 to 147 (ng/ml)/(mg/kg), that was also observed throughout the first 6 months, as demonstrated by the between-subject percent coefficient of variation ranging from 37% at day 90 up to 59% at day 30 (Fig. 1a). The type of adjunctive therapy did not affect between-subject variability at any time after transplantation ($P>0.05$).

We made daily cyclosporine dose adjustments in 23 patients in an attempt rapidly to achieve therapeutic drug concentrations. Mean cyclosporine doses were increased from 520 ± 78 mg/day at day 1 to 522 ± 78, 567 ± 98, 598 ± 111, 620 ± 120, 624 ± 150, and

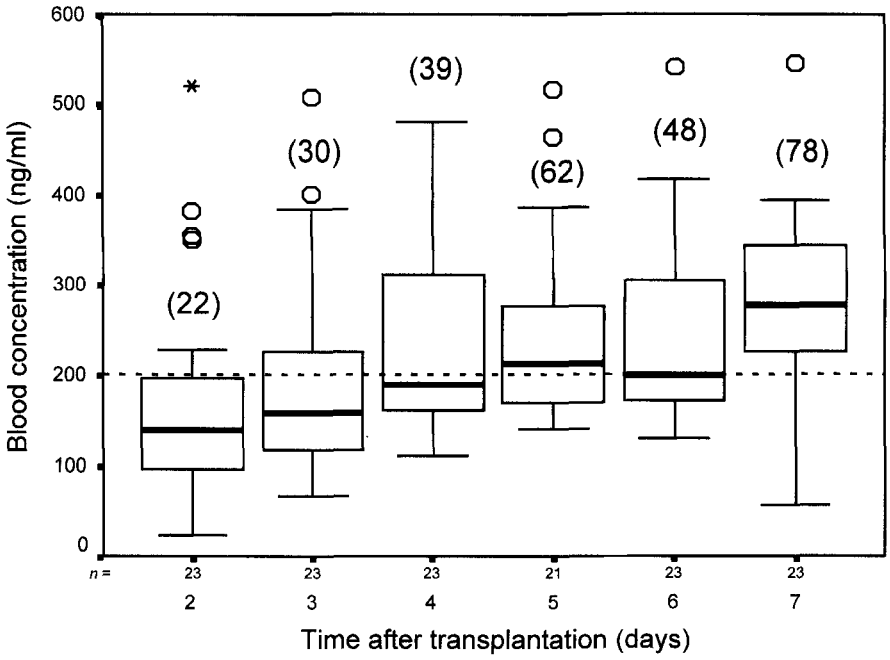

Fig. 2 Distribution of cyclosporine blood concentrations during the first week after transplantation ($n=23$). *Open circles* represent outliers and *asterisk* extreme values. *Numbers in parenthesis* are the percentage of patients with cyclosporine blood concentrations above 200 ng/ml; $P=0.001$, Wilcoxon signed rank test comparing days 2 to 7

617 ± 164 mg/day at days 2, 3, 4, 5, 6, and 7 after transplantation, respectively ($P<0.001$). Compared with day 2, when first blood concentration was determined, cyclosporine doses were increased by 19% at day 7 ($P=0.02$). Mean blood cyclosporine concentrations increased from 173 ± 120 at day 2 to 196 ± 114, 227 ± 106, 246 ± 105, 248 ± 108, and 283 ± 101 ng/ml at days 3, 4, 5, 6, and 7, respectively ($P<0.001$). Mean dose-adjusted blood concentrations increased from 0.69 ± 0.48 ng/ml per mg at day 2 to 0.76 ± 0.45, 0.82 ± 0.40, 0.84 ± 0.37, 0.86 ± 0.51, and 1.0 ± 0.50 ng/ml per mg at days 3, 4, 5, 6, and 7, respectively ($P=0.036$; Fig. 1b). Between-subject variability decreased from 70% at day 2 to 51% at day 6 (Fig. 1b; $P<0.001$). The percentage of patients with blood cyclosporine concentrations above 200 ng/ml increased from 22% at day 2 to 30, 39, 62, 48, and 78% at days 3, 4, 5, 6, and 7, respectively ($P<0.001$; Fig. 2).

Within-subject variability in dose-adjusted blood cyclosporine concentrations was higher during the first 30 days than that observed between 60 and 180 days after transplantation. Median %CV for C0 concentrations were 29% (13–77%) between days 2 and 7, 32% (8–84%) between days 8 and 30, and 22% (3–56%) between days 60 and 180 (Fig. 3; $P=0.008$). There was no correlation between within-subject variability and therapeutic cyclosporine concentrations at day 3 ($P=0.28$). Within pharmacokinetic studies, less variability was observed when AUC and cyclosporine concentrations (C0, Cmax, C12) were compared. Comparison of the data obtained at months 2, 3, and 6 showed within-subject variability (%CV) of 13.6% (0.3–49.5%) for AUC, 19.8% (1.1–59.2%) for Cmax, 15.9% (6.8–85.1%) for C0, and 26.6% (2.9–73.2%) for C12 (Fig. 3; $P=0.036$).

Concentration-efficacy correlations

The overall incidence of biopsy-proven acute rejection was 20.5% (14/68). Acute rejection episodes occurred at a mean time of 14.9 ± 12 days, ranging from 2 to 36 days after transplantation. Mean trough blood cyclosporine concentrations at day 3 did not differ between patients with or without rejection (201 ± 143 vs 200 ± 102 ng/ml, $P>0.05$). Mean trough blood cyclosporine concentration at the time of rejection was 238 ± 150 ng/ml, being below 200 ng/ml in six (43%) patients. The incidence of acute rejection did not differ between patients with trough blood cyclosporine concentration below or above 200 ng/ml at day 3 (20% vs 21%, $P>0.05$, respectively). Using logistic regression analysis, we observed that ethnicity (OR = 0.61), HLA mismatch (OR = 0.92), adjunctive therapy (OR = 0.98), or blood cyclosporine concentrations below 200 ng/ml at day 3 (OR = 0.99) were not associated with the occurrence of acute rejection.

Discussion

Rapid obtainment and maintenance of target and stable cyclosporine exposure appear to reduce both acute and chronic kidney allograft rejection [18, 20, 21, 29, 32, 33, 34, 36]. Although the cyclosporine microemulsion has

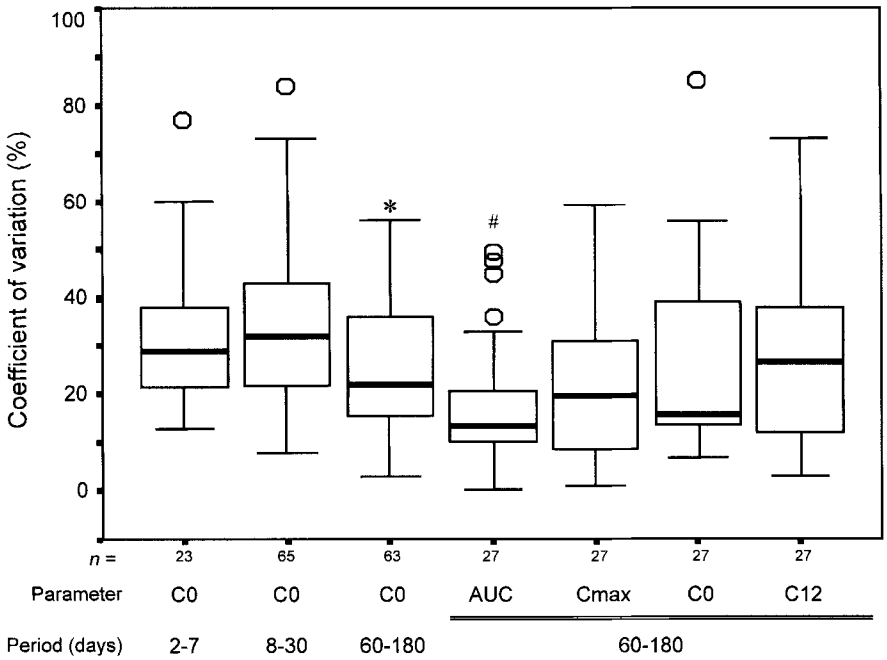

Fig. 3 Within-subject variability in cyclosporine exposure parameters obtained after transplantation. Distribution of percent coefficients of variation (%CV) of mean dose-adjusted cyclosporine blood concentrations or dose-adjusted pharmacokinetic parameters in three periods after transplantation. *Open circles* represent outliers. $^{*}P = 0.008$, Wilcoxon signed rank test comparing C0 values at periods 2–7, 8–30, and 60–180 days after transplantation. $^{\#}P = 0.0036$, Wilcoxon signed rank test comparing AUC, Cmax, C0, and C12

shown pharmacokinetic advantages over the oil-based formulation (Sandimmun), the between-subject variability and narrow therapeutic index still require its therapeutic monitoring [4, 24, 42]. Difficult to achieve, target drug exposure early after transplantation is still a problem for a considerable fraction of patients and may be due to patient-related and/or drug-related factors [34]. The absorption of orally delivered drugs is generally reduced after transplant surgery, depending on the type and duration of anesthesia, hydration, and reduced intestinal motility [12]. Although the microemulsion formulation has minimized the poor solubility and high lipophilicity of cyclosporine and the release of the drug from the formulation, the window of absorption in the small intestine, and differences in content and activity of CYP450 3A4 and P-glycoprotein may still significantly influence the absorption of cyclosporine from the microemulsion formulation [13, 28, 30, 39]. Therefore, higher initial cyclosporine doses during the first week than those used from the second week on after transplantation are required if therapeutic concentrations are to be achieved. In a cohort of 23 patients, the initial cyclosporine dose used was sufficient to achieve target cyclosporine concentrations at day 3 in only 30% of the patients. Repeated increases of 100 mg/day in cyclosporine doses, from days 2 to day 7 after transplantation, increased the number of patients with therapeutic concentrations to 78% by day 7. Between days 2 and 7, the dose increment of 19% (522 ± 78 to 617 ± 164 mg/day) was followed by a 64% increase in blood cyclosporine concentrations (173 ± 120 to 283 ± 101 ng/ml) and a 45% increment in dose-adjusted cyclosporine concentrations (0.69 ± 0.48 to 1.0 ± 0.5 ng/ml per mg), respectively. Among 65 patients receiving mean cyclosporine doses of 527 ± 108 mg/day at day 3, 63% showed cyclosporine concentrations below 200 ng/ml. Between days 8 and 15, doses were reduced by 10% (577 ± 130 to 520 ± 142 mg/day), but increases of 20% in blood cyclosporine concentrations (308 ± 150 to 370 ± 131 ng/ml) and of 36% in dose-adjusted cyclosporine concentrations (1.1 ± 0.6 to 1.5 ± 0.6 ng/ml per mg) were observed. Maximum mean dose-adjusted cyclosporine concentration of 2.0 ± 1.2 ng/ml per mg was reached by day 30, when cyclosporine doses had been reduced by 33% but blood cyclosporine concentrations had increased by 11% (308 ± 150 to 344 ± 137 ng/ml) compared to day 8. Although the disproportional increase in dose-adjusted cyclosporine concentrations observed during the first week may be predominantly related to drug accumulation after multiple dose administration, the further increase observed between days 8 and 30 is probably related to time-dependent changes in relative cyclosporine absorption, since cyclosporine doses were reduced and blood measurements were all done at steady state. From day 60 to day 180, no differences were found in dose-adjusted blood cyclosporine concentrations. Translating these findings to clinical practice, we observed a wide range of dose-adjusted cyclosporine concentrations at day 3, from 14 to 147 (ng/ml)/(mg/kg per dose). This suggests that the initial cyclosporine doses of 4–5 mg/kg per dose (8–10 mg/kg per day) would be sufficient to produce therapeutic concentrations (200 ng/ml) for only 31–58% of the patients at day 3. In fact, about 15% would need 7 mg/kg per dose (14 mg/kg per day) and 3% 10 mg/kg per dose (20 mg/kg per day).

The pharmacokinetic studies revealed similar results. Dose-adjusted pharmacokinetic parameters were significantly lower (~50%) at day 4 than those observed at 2, 3, and 6 months. No differences were observed when we compared C0, Cmax, C12, AUC, or Cavg obtained at day 4 or month 2, although doses were 88% higher at day 4. From month 2 to month 6, no differences were observed on dose-adjusted pharmacokinetic parameters, which suggests that pharmacokinetic stability had been achieved by month 2, as previously demonstrated by Kovarik et al. [26].

Altogether, these findings strongly suggest that the increase in dose-adjusted cyclosporine exposure parameters observed during the first month after transplantation is related to time-dependent increases in relative bioavailability of cyclosporine [2, 11]. It is unlikely that the magnitude of differences observed in dose-adjusted cyclosporine pharmacokinetic parameters is exclusively due to lack of dose–concentration linearity at cyclosporine doses used early after transplantation. Moreover, the changes in pharmacokinetics did not correlate with the increase in hemoglobin over time ($r^2 = 0.02$, $P > 0.05$, data not shown), as demonstrated by Awni et al. for the oil-based cyclosporine formulation [2] but not confirmed by Kovarik et al. for the microemulsion formulation [26].

The mechanisms involved in this effect are not completely understood [2, 11]. In rats, blood cyclosporine concentrations increase following chronic daily administration and are paralleled by a decrease in hepatic P450 protein expression and microsomal metabolic activity, which suggests that time-dependent P450 suppression by cyclosporine may explain, at least in part, the observed time-dependent changes in cyclosporine pharmacokinetics [3, 5, 26]. Regardless of the mechanisms responsible for these changes or the strategy used to monitor the clinical use of cyclosporine (trough, C2, or abbreviated AUC), it is clear that higher initial cyclosporine doses are required if therapeutic concentrations early after transplantation are to be produced. This fact can be complicated if even higher initial exposures are necessary early after transplantation to prevent acute rejection, as suggested by Mahalati et al. [34]. In that study, in which intensive monitoring was used to reach a specific therapeutic window early after transplantation, only 60% of the patients reached a therapeutic window of AUC0-4 higher than 4400 ng×h/ml on postoperative day 3, and the cyclosporine doses required to reach the target exposure were not mentioned [34].

Between-subject variability in cyclosporine C0 concentrations was reduced after repeated doses of cyclosporine during the first week of transplantation and did not change thereafter till day 180 (Fig. 1). Between-subject variability within pharmacokinetic studies was low-to-moderate and did not change when day 4 was compared with months 2, 3, and 6 after transplantation. Within-subject variability was analyzed during periods of unstable (2–7 days or 8–30 days) or stable (60–180 days) cyclosporine pharmacokinetics. Within-subject variability decreased over time after transplantation from 29% between days 2 and 7 to 22% between 60 and 180 days. Within-subject variability was even lower when assessed by AUC (13.6%) or Cmax (15.9%) within pharmacokinetic studies. These results indicate that the initial period of transplantation, when cyclosporine pharmacokinetics are still unstable, is associated with higher between-subject and within-subject variability, a fact that may complicate the adjustment of cyclosporine dose to gain rapid achievement and maintenance of therapeutic concentrations.

Two main pharmacokinetic reasons justify the use of peak (C2) over trough (C0 or C12) cyclosporine monitoring. First, there is good and consistent correlation between C2 and AUC during the first 6 months after transplantation (Table 3). Second, there is lower intra-subject variability of peak cyclosporine concentrations (Fig. 3). Since both between-subject and within-subject variability appears to be higher for C0 or C12 than for Cmax or AUC, it may be easier to find the cyclosporine dose which confers target blood exposure early after transplantation based on C2 or AUC measurements. Moreover, the estimation of variability of oral absorption of cyclosporine based on serial C0 determinations over time in an attempt to identify patients at higher risk of developing chronic allograft rejection may overestimate the population at higher risk, compared with true measurement of cyclosporine exposure by AUC. Since the AUC that was calculated from combinations of three early cyclosporine concentrations showed good correlation with total AUC (Table 3), abbreviated strategies may be more useful, either early or in the maintenance phase of transplantation [9, 18, 19]. In fact, a simple measurement of C2 may be sufficient, according to recent data [4, 15].

The overall incidence of acute rejection was low and was associated with cyclosporine concentrations below the target range at the time of rejection, but not at day 3. The lack of association between cyclosporine concentration at day 3 and the incidence of acute rejection might be related to many factors. The small number of patients in this study as well as the low and inconsistent correlation between C0 and exposure or low positive predictive value for rejection of trough cyclosporine concentrations may be involved [34]. Another possibility would be the type of adjunctive therapy that was used, since subgroups of patients received drugs (MMF, EVL, and FTY) that were supposedly more effective than AZA. However, logistic regression analysis ruled out this possibility.

Based on these findings, initial cyclosporine doses higher than the typical 8–10 mg/kg per day and early dose adjustments may be necessary to achieve higher

cyclosporine exposures, which have been associated with reduced incidence of acute rejection [34]. Whether intense cyclosporine monitoring or synergistic adjunctive therapy, or both, have to be used to reduce acute rejection rates further is not known. It appears attractive for one to use an anti-interleukin-2 receptor antibody and/or a proliferating signal inhibitor, such as sirolimus or EVL, to compensate for early low, variable, and unstable cyclosporine exposure. In fact, acute rejection rates when these combinations are used are very low under routine cyclosporine therapeutic monitoring [8, 16, 31]. Also, whether the long-term use of new immunosuppressive agents will minimize or compensate for the impact of intra-subject variability in cyclosporine exposure on the incidence of chronic allograft rejection is still unknown.

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