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ORIGINAL ARTICLE

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Cyclosporine absorption profiles in pediatric kidney and liver transplant patients

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Abstract Cyclosporine absorption profiling uses either the area under the concentration curve in the first 4 h post dose, AUC(0–4), or the concentration 2 h post dose (C2) to optimize immunosuppression in adult kidney and liver transplantation. We characterized C2 versus AUC(0–4) relationships over time after transplant and across transplant indications in 56 pediatric transplant patients. There were 36 kidney transplant patients aged 9.7±3.9 years. Nineteen of these patients were studied in the de novo period on day 7 post transplant and 17 in the maintenance phase more than 1 year post transplant. In addition, 20 liver transplant patients aged 8.9±4.2 years were studied in the maintenance phase. All patients had five blood samples collected over the 12-h dose interval that were analyzed by validated assay methods at a central laboratory. Pediatric C2 values were 1,463±658 ng/ml for de novo kidney, 954±322 ng/ml for maintenance kidney, and 619±339 ng/ml for maintenance liver transplant patients. C2 was a strong predictor of AUC(0–4) in all three pediatric groups, with coefficients of determination (r^2) ranging from 0.861 to 0.936. Although data were limited from the de novo period, the C2 versus AUC(0–4) regression was consistent over time after transplant and between transplant indications, with a regression slope of 2.50 in de novo kidney, 2.54 in maintenance kidney, and 2.76 in maintenance liver transplant recipients. These slopes were also comparable to that in adult maintenance kidney transplant patients (2.60). In conclusion, C2 versus AUC(0–4) relationships demonstrated consistency over time (de novo vs. maintenance phase), between transplant indications (kidney vs. liver), and across age groups (pediatric vs. adult patients). Average C2 values achieved with current pediatric cyclosporine dosing practices cluster around the target C2 ranges recommended for adults.

Keywords Cyclosporine · Therapeutic drug monitoring · Kidney transplantation · Liver transplantation · Immunosuppression

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Introduction

Cyclosporine is a core component used in immunosuppressive regimens for pediatric organ transplants [1]. In both pediatric and adult organ transplant patients, cyclosporine dosing is guided by therapeutic drug monitoring, and similar trough target blood levels are used in the two age groups [2]. In comparison with adults, children generally require higher doses on a milligram per kilogram basis. Although the introduction of a microemulsion formulation of cyclosporine (Neoral, Novartis Pharma) has largely corrected absorption problems in children, there remain differences in bioavailability [3] and clearance, especially in young children [4].

Until recently, monitoring was based on pre-dose (trough) blood levels. The current focus in adult transplantation is on the early portion of the cyclosporine area under the concentration-time curve, particularly in the first 4 h post dose, designated as AUC(0–4). This region constitutes a major portion of the total AUC over the 12-h dosing interval, accounts for a large share of the between-patient differences in exposure, and is the period in which calcineurin inhibition in lymphocytes (the pharmacodynamic effect) is maximal [5]. Indeed, cyclosporine

AUC(0–4) can identify patients who are low, intermediate, or high absorbers of cyclosporine, allowing their dose to be adjusted accordingly, and it serves as a significant predictor of acute rejection in adult renal and liver transplantation [5]. Given the clinical burden of blood sampling over a 4-h interval to characterize AUC(0–4), clinicians have sought a single-point marker to apply in therapeutic drug monitoring. Several studies have consistently shown that the cyclosporine blood concentration 2 h post dose (C2) is a highly predictive marker for AUC(0–4). Adjusting cyclosporine doses to achieve target C2 levels ("C2 monitoring") has resulted in remarkably low rejection rates, without increased tolerability issues in de novo recipients of liver [6] and renal [7] grafts, and in safe dose reductions for >30% of liver or kidney recipients in the maintenance phase [8, 9]. An international consensus statement with recommended target concentrations [10] and an implementation strategy [11] have been issued.

Few clinical data exist on cyclosporine C2 or AUC(0–4) in pediatric organ transplant patients. Hoyer and Vester [12] have reported 36 pediatric kidney transplant patients in whom cyclosporine blood concentrations were obtained at 0, 1, 2, 3, and 4 h post dose. C2 was the best single-point predictor of AUC(0–4), with a coefficient of determination (r^2) of 0.99, clearly outperforming C0 (r^2=0.56) . Dunn et al. [13] retrospectively assessed C2 and C0 levels in 26 maintenance pediatric liver transplant patients and prospectively in 9 patients in the de novo period. They reported a strong correlation between C2 and AUC(0–4) in both groups (r^2=0.89 and 0.93, respectively) with poorer correlations for C0 and AUC(0–4) (r^2=0.03 and 0.53, respectively). While these data confirm C2 as a good single-point marker for AUC(0–4) in children, no information has yet been published as to whether a given C2 level corresponds to a similar AUC(0–4) in children as it does in adults.

In an effort to generate more information on cyclosporine C2 and AUC(0–4) in pediatric transplant patients, we analyzed data from three recent clinical studies with a total of 56 pediatric patients. We explored whether cyclosporine C2 and AUC(0–4) exposure in pediatric patients were comparable to those in adults, whether the correlation between C2 and AUC(0–4) differed between pediatric renal and liver transplant patients, and whether this correlation was preserved over time post transplant. The ultimate aim of this evaluation was to lay a foundation for prospective, controlled trials to validate C2 monitoring in pediatric patients.

Materials and methods

Studies and patients

Cyclosporine blood sampling was prospectively performed in three multicenter clinical studies during the pediatric clinical development of everolimus [assessments in adults have shown that everolimus does not influence the cyclosporine C2 vs. AUC(0–4) relationship]. The pediatric de novo renal transplant study enrolled 19 patients whose immunosuppressive regimen consisted of cyclosporine microemulsion (Neoral, Novartis), everolimus (Certican, Novartis), and corticosteroids [14]. The cyclosporine profile was obtained on day 7 post transplant. The pediatric maintenance renal transplant study enrolled 17 patients receiving cyclosporine microemulsion and corticosteroids [15]. They received a single dose of everolimus on study day 1, at which time the steady-state cyclosporine pharmacokinetic profile was obtained. The pediatric maintenance liver transplant study was identical and enrolled 22 patients [16]. These three studies were conducted in 2000 and 2001 at a total of 16 kidney transplant centers and 8 liver transplant centers in Europe and North America. The study protocols were approved by ethical review committees at each study center. Parents or guardians gave informed consent, and patients, depending on their age, gave informed assent to participate in the trials.

Pharmacokinetic assessments

Cyclosporine pharmacokinetic profiles were obtained over a 12-h dose interval at steady state. Blood samples were drawn into EDTA-containing collection tubes pre dose and then 1, 2, 5, 8, and 12 h post dose. Sample tubes were inverted several times to mix the blood with anticoagulant and then frozen at −20°C. Cyclosporine blood concentrations were determined by a liquid chromatography method coupled with mass spectrometry in the de novo and maintenance kidney transplant studies. The analytical method has been previously described and had lower limits of quantification in these studies of 7 and 9 ng/ml [17]. In the maintenance liver transplant study, cyclosporine was analyzed with a commercially available radioimmunoassay (Incstar Cyclo-Trac, Diasorin, Stillwater, Minn., USA) based on a monoclonal antibody specific for the parent compound. The assay was performed according to the manufacturer's instructions and had a lower limit of quantification of 50 ng/ml in this study. The biochemical analyses for all three studies were performed at a central laboratory.

Data analysis

Standard non-compartmental pharmacokinetic parameters were generated with WinNonlin (version 3.2, Pharsight, Mountain View, Calif., USA). These included the pre-dose trough concentration C0, the concentration 2 h post dose C2, the maximum concentration Cmax, and the AUC over the dose interval AUC(0–12). The AUC(0–4) was estimated in WinNonlin with the partial AUC algorithm included in this software program. As applied to these data, the algorithm calculated the AUC to 2 h post dose based on the measured data at C0, C1, and C2, and then added the additional contribution between C2 and C4 by linear interpolation of the data between the measured values at C2 and C5. The correlation between C2 or C0 versus AUC(0–4) was assessed by conventional linear regression and the corresponding coefficient of determination (r^2 value). Data are expressed as mean±standard deviation unless otherwise noted.

We checked whether the interpolation procedure used in this study biases the pharmacokinetic results based on previously collected data in 25 adult renal transplant patients who had hourly blood sampling. The AUC(0–4) calculated from concentrations at 0, 1, 2, and 4 h post dose was 1874±638 ng h/ml compared with an AUC(0–4) from concentrations at 0, 1, 2, and 5 h (with the 4-h concentration interpolated) of 1822±615 ng h/ml. The AUC(0–4) values between the two approaches were highly correlated (r^2=0.998) with the AUC(0–4) from interpolation being only 3% lower than the area without interpolation.

Results

Patients

Table 1 summarizes the demographic characteristics of the study patients. While few infants (<2 years) were enrolled, there was a good distribution across the age range of children (2–11 years) and adolescents (12–16 years) in all three studies. Weight ranged from 10.7 to 76.8 kg and body surface area from 0.49 to 1.92 m^2. Of the total 56 patients, there were 35 boys and 21 girls. The ethnic groupings were typical for a population derived from European and American transplant centers: 38 whites, 10 blacks, and 8 of other ethnicities. The de novo transplant patients were studied at 1 week post transplant and the maintenance patients were studied at least 1 year after transplantation.

Cyclosporine dosing and exposure in pediatric renal and liver allograft recipients

Table 2 summarizes the derived pharmacokinetic parameters. In the de novo renal transplant study, the protocol-specified cyclosporine regimen began with a dose of 6–12 mg/kg per day in two divided doses every 12 h, which was then individualized to achieve target C0 concentrations between 200 and 350 ng/ml in the 1st post-transplant month. When the pharmacokinetic profile was obtained on day 7, patients were receiving doses of 13.1±3.2 mg/kg per day, corresponding to 360±57 mg/m^2 per day. The average C0 of 215±124 ng/ml was at the lower end of the target window, with individual values ranging from 77 to 498 ng/ml across the patients. C2 averaged 1,463±658 ng/ml, with individual values ranging from 430 to 2650 ng/ml. Figure 1 places these C2 values in the perspective of the recommended adult C2 target for the 1st post-transplant month of 1,500–2,000 ng/ml [11].

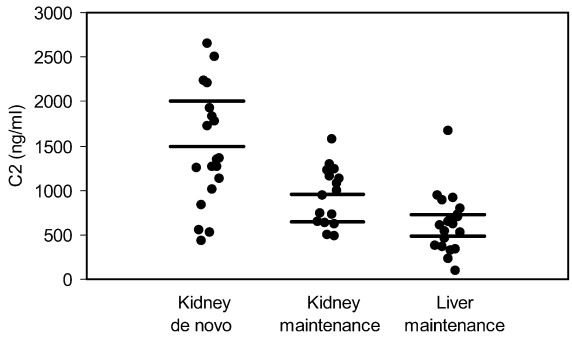

Fig. 1 Individual cyclosporine blood concentrations 2 h post dose (C2) in pediatric patient groups. The *bars* represent the recommended C2 range (target concentration±20%) in adult patients according to transplanted organ and time after transplantation. Cyclosporine in the pediatric patients was dosed based on pre-dose trough (C0) blood level monitoring

Maintenance renal transplant patients were receiving roughly half the dose of the de novo patients, averaging 6.7±2.5 mg/kg per day or 189±62 mg/m^2 per day. C0 averaged 142±50 ng/ml, with a range from 68 to 224 ng/ml. The corresponding C2 value was 954±322 ng/ml and ranged from 482 to 1,570 ng/ml. As depicted in Fig. 1, the pediatric C2 concentrations were clustered about the recommended adult target of 800 ng/ml after 1 year post transplant [11].

Table 1 Pediatric study populations

Characteristic	De novo kidney	Maintenance kidney	Maintenance liver
n	19	17	20
Age (years)	9.9±4.4	9.0±3.4	8.9±4.2
Age category			
Infant (<2 years)	1	0	1
Child (2–11 years)	10	12	14
Adolescent (12–16 years)	8	5	7
Weight (kg)	32.7±19.7	31.5±14.9	31.7±13.1
Body surface area (m^2)	1.06±0.43	1.05±0.33	1.07±0.31

Table 2 Cyclosporine pharmacokinetics (*Cx* concentration *x* hours post dose, *Cmax* maximum concentration, *AUC(0-x)* area under the blood concentration-time curve from 0 to *x* hours post dose, r^2 coefficient of determination from linear regression)

Parameter	Pediatric de novo kidney	Pediatric maintenance kidney	Adult maintenance kidney	Pediatric maintenance liver
n	19	17	173	20
Dose (mg/kg per day)	13.1±3.2	6.7±2.5	–	5.7±3.3
C0 (ng/ml)	215±124	142±50	181±108	127±49
C2 (ng/ml)	1,463±658	954±322	883±417	619±339
Cmax (ng/ml)	1,821±631	1,286±326	1,077±429	657±321
AUC(0–4) (ng h/ml)	4,734±1,733	3,247±882	2,772±1,173	1,822±967
AUC(0–12) (ng h/ml)	7,871±2,351	4,971±1428	4,630±1,788	3,089±1,581
Intercept (ng h/ml)	1,077	821	475	111
Slope	2.50	2.54	2.60	2.76
r^2	0.900	0.861	0.854	0.936

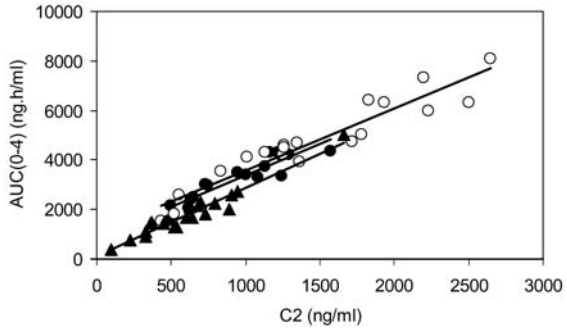

Fig. 2 Regression of the cyclosporine concentration 2 h post dose (C2) and the area under the concentration-time curve in the first 4 h post dose [AUC(0–4)] in pediatric de novo kidney (*open circles*), maintenance kidney (*filled circles*), and maintenance liver (*filled triangles*) allograft recipients. Shown are the associated regression lines. The corresponding regression parameters are listed in Table 2

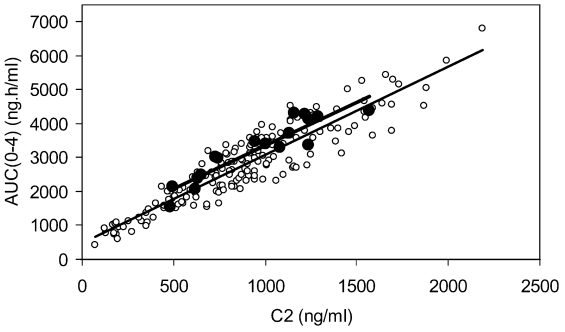

Fig. 3 Regression of the cyclosporine concentration 2 h post dose (C2) and the area under the concentration-time curve in the first 4 h post dose [AUC(0–4)] in pediatric (*filled circles*) and adult (*open circles*) maintenance kidney allograft recipients. Shown are the corresponding regression lines. The pediatric data are the same as in Fig. 2 for maintenance kidney transplant patients and serve as a link between the figures

Maintenance liver transplant patients received cyclosporine doses of 5.7±3.3 mg/kg per day, corresponding to 155±68 mg/m^2 per day. C0 values averaged 127±49 ng/ml, with a range from 52 to 243 ng/ml. The C2 value was 619±339 ng/ml (range 92–1,662 ng/ml) and was near the recommended adult target of 600 ng/ml after the first 6 months post transplant (Fig. 1).

C2 correlations in pediatric renal and liver transplant patients

As shown in Table 2 and Fig. 2, there were strong correlations between C2 and AUC(0–4) in both pediatric de novo and maintenance renal transplant patients, with coefficients of determination (r^2) of 0.900 and 0.861, respectively. Regression slopes were remarkably consistent between populations and time after transplantation. In both cases, C0 was a notably poor predictor of AUC(0–4), with an r^2 of 0.054 and 0.522.

In pediatric liver transplant patients, the C2 to AUC(0–4) regression was very similar to those in pediatric renal transplant patients, as shown in Fig. 1. Again, C2 was a better predictor of AUC(0–4) than C0, as demonstrated by the r^2 of 0.936 and 0.156, respectively.

Comparative adult renal transplant data

Adult cyclosporine data were collected from the two international everolimus phase 3 trials in kidney transplantation in which 173 patients had steady-state AUC profiles obtained at 6 months post transplant [18]. Firstly, we divided the data between patients receiving cyclosporine with mycophenolate mofetil and corticosteroids (n=82) and those receiving cyclosporine with everolimus and corticosteroids (n=91) to determine if the presence of everolimus in the immunosuppressive regimen influenced the cyclosporine C2 to AUC(0–4) correlation. The slopes (2.62 and 2.60), intercepts (547 and 401 ng h/ml), and r^2 values (0.848 and 0.867) were similar in patients receiving mycophenolate mofetil and those receiving everolimus.

We next pooled the adult data across all 173 patients to compare with the data from pediatric maintenance patients as summarized in Table 2 and Fig. 3. Apart from a modestly lower intercept, the slopes were nearly identical between the adult and pediatric regressions.

Discussion

Pharmacokinetic and clinical data in adult renal and hepatic transplantation indicate that cyclosporine immunosuppression can be improved in terms of acute rejection prophylaxis and renal safety when Neoral dose is based on C2 therapeutic monitoring compared with C0 monitoring [5]. Given the paucity of pharmacokinetic data in pediatric transplantation, we retrospectively analyzed data in 56 pediatric transplant patients gathered during recent immunosuppressive drug development trials using cyclosporine-based regimens.

Although our evaluation was retrospective, the data were well suited to the goals of our analysis. Firstly, the data were derived from a demographically and geographically heterogeneous patient population, representing the full range of pediatric age, weight, and body surface area, along with a good mix of ethnicities and a near balance between genders. Secondly, the data were from independent populations in terms of time after transplantation (de novo versus maintenance phases) and transplant indication (kidney versus liver). Both the heterogeneity and independence of the study populations allowed a robust assessment of the C2 versus AUC(0–4) regression. Thirdly, the data were collected from pediatric populations whose cyclosporine dosing was guided by the current standard of practice for therapeutic monitoring in this population using C0. This allowed us to characterize the C2 concentrations pediatric patients are currently

achieving, to serve as a reference for studies implementing prospective C2 monitoring in this population.

We noted that cyclosporine C2 was strongly correlated with drug exposure in the absorption region of the area under the concentration-time curve, namely AUC(0–4). This is in agreement with the single-center observations of Dunn et al. [13] who assessed this in pediatric liver transplant recipients and of Hoyer and Vester [12] and of Trompeter et al. [3] in pediatric renal transplant patients. Furthermore, the regressions were similar between pediatric kidney allograft patients on day 7 after transplant and during the maintenance period, as well as between pediatric maintenance renal and maintenance liver allograft recipients. The C2 values achieved in pediatric patients with current cyclosporine dosing practices were near those recommended for adult patients, according to transplanted organ and time post transplant [11].

Our comparison of pediatric and adult maintenance kidney transplant patients demonstrated that a given C2 value in a pediatric patient corresponds to a similar AUC(0–4) exposure, as it does in adult patients (Fig. 3). The pediatric pharmacokinetic data in the first weeks after transplantation were limited in our analysis. Given the wider scatter of C2 values on day 7 compared with the maintenance phase (Fig. 2), more data are needed to better characterize the comparability and stability of the C2 to AUC(0–4) relationship in the early post-transplant weeks. The importance of attaining adequate cyclosporine exposure in this critical 1st week has been shown in adults [5] and is underscored by Trompeter et al. [3] in pediatric patients. Based on a longitudinal assessment of cyclosporine pharmacokinetics over 6 months after kidney transplantation, these investigators noted retrospectively that patients achieving C2 >1,500 ng/ml by the 5th postoperative day experienced no acute rejections compared with a 50% rejection rate in patients with C2 below this level. Renal function did not appear to be adversely influenced by C2 levels [3].

Our descriptive evaluation of cyclosporine pharmacokinetics, together with the observations of the clinical relevance of C2 levels in pediatric patients [3], provide a necessary foundation for initiation of prospective clinical trials in pediatric patients. Such trials are needed to assess in a controlled manner whether C2 monitoring can be implemented in pediatric populations, whether this approach can prospectively improve the efficacy and safety of cyclosporine-based immunosuppressive regimens, and whether the C2 target concentrations used in adults are appropriate across the pediatric age range.

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