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Sirolimus Pharmacokinetics in Early Postmyeloablative Pediatric Blood and Marrow Transplantation

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Abstract

This study examined the pharmacokinetics of sirolimus in pediatric allogeneic blood and marrow transplantation (BMT) recipients in the presence and absence of concomitant fluconazole. Forty pediatric BMT recipients received a daily oral dose of sirolimus and a continuous i.v. infusion of tacrolimus for graft-versus-host disease prophylaxis. Fluconazole was administered i.v. to 19 patients and orally to 6 patients. Full pharmacokinetic profiles of sirolimus within a single dosing interval were collected. Whole-blood sirolimus concentrations were measured by HPLC/mass spectrometry. Noncompartmental analysis was performed using WinNonlin. Nonlinear mixed-effects pharmacokinetic models were developed using NONMEM following standard procedures. The mean \pm SD sirolimus trough level before the dose (C_0) was 8.0 ± 4.6 ng/mL (range, 1.8–21.6 ng/mL). The peak concentration was 19.9 ± 11.8 ng/mL (range, 3.9–46.1 ng/mL), and the trough level 24 hours later (C_{24}) was 9.1 ± 5.3 ng/mL (range, 1.0–19.1 ng/mL). The terminal disposition half-life ($T_{1/2}$) was 24.5 ± 11.2 hours (range, 5.8–53.2 hours), and the area under the concentration-versus-time curve (AUC_{0-24}) was 401.1 ± 316.3 ng·h/mL (range, 20.7–1332.3 ng·h/mL). In patients at steady state, C_0 and C_{24} were closely correlated ($R^2 = 0.77$) with a slope of 0.99, indicating the achievement of steady state. C_{24} was 1.7-fold greater ($P = .036$) and AUC_{0-24}

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was 2-fold greater ($P = .012$) in Caucasian patients ($n = 22$) compared with Hispanic patients ($n = 9$). The average apparent oral clearance was 3-fold greater ($P = .001$) and the apparent oral volume of distribution was 2-fold greater ($P = .018$) in patients age ≤ 12 years compared with those age >12 years. C_{24} was significantly lower in patients ($n = 10$) who developed grade III–IV aGVHD ($n = 10$) than in those with grade 0–II aGVHD ($n = 22$) (6.1 ± 2.9 ng/mL versus 9.4 ± 5.5 ng/mL; $P = .044$). Dose-normalized sirolimus trough concentrations were significantly higher in patients receiving concomitant fluconazole therapy compared with those not receiving fluconazole (C_0 : 3.9 ± 2.5 versus 2.4 ± 1.5 ng/mL/mg, $P = .030$; C_{24} : 4.8 ± 3.3 versus 2.5 ± 1.7 ng/mL/mg, $P = .018$). This pharmacokinetic study of sirolimus in pediatric patients documents a large interindividual variability in the exposure of sirolimus. Steady-state trough blood concentrations were correlated with drug exposure. Trough concentrations were higher with a concomitant use of fluconazole and were higher in Caucasian patients than in Hispanic patients. Oral clearance was greater in children age ≤ 12 years than in older children and adolescents. With therapeutic drug monitoring, the majority (79%) of sirolimus trough levels could be maintained within the target range (3–12 ng/mL). This study provides a rationale and support for dose adjustments of sirolimus based on steady-state blood concentrations aimed at achieving a target concentration to minimize toxicity and maximize therapeutic benefits in pediatric BMT recipients.

Keywords

Graft-versus-host disease; Fluconazole; Population pharmacokinetic analysis; Therapeutic drug monitoring

INTRODUCTION

Sirolimus (or rapamycin) is a macrocyclic antibiotic with immunosuppressive and antineoplastic properties. It binds to the FK-binding protein 12 and inhibits the mammalian target of rapamycin, resulting in cell cycle arrest at the G1/S phase transition and a suppression of cytokine-mediated T cell proliferation, a mechanism of action distinct from that of tacrolimus.

In solid organ transplant recipients, sirolimus and tacrolimus have been used in combination owing to their synergistic immunosuppressive effects and nonoverlapping toxicity profiles. In blood and marrow transplantation (BMT) recipients, sirolimus has been used for the prevention and treatment of graft-versus-host disease (GVHD) [1,2]. In adult BMT recipients receiving myeloablative conditioning, a prophylactic regimen including sirolimus and tacrolimus was associated with lower rates of acute GVHD (aGVHD) and less mucositis compared with a regimen including tacrolimus and methotrexate [2].

Sirolimus is available only as an oral formulation and has low bioavailability owing to countertransport into the gut lumen by the p-glycoprotein (P-gp) multidrug efflux pump and extensive first-pass metabolism in the intestinal wall and the liver. Numerous factors can influence the absorption and bioavailability of sirolimus in BMT recipients, including conditioning-related nausea and emesis, mucositis and intestinal GVHD, functional gene polymorphisms in metabolic enzymes, and the concurrent use of CYP450 inhibitors, such as azole antifungal drugs [3,4].

Fluconazole is widely used for *Candida* prophylaxis in patients undergoing BMT who receive cytotoxic chemo-radiotherapy. The literature on interactions between fluconazole and sirolimus is scant, but such interactions have been implied largely from 2 case reports and based on observed interactions between other azoles and calcineurin inhibitors [5–7].

Because of its long half-life, sirolimus is administered once a day to achieve the target therapeutic concentrations. Pharmacokinetic studies have shown a shorter half-life in pediatric solid organ transplant recipients compared with healthy controls and adult transplant recipients [8,9]. Although the pharmacokinetics of sirolimus have been well studied in solid organ transplant recipients, the drug has not yet been thoroughly evaluated in pediatric BMT recipients.

We recently reported clinical findings from a multi-institutional pilot trial of the addition of sirolimus to tacrolimus-methotrexate GVHD prophylaxis in children undergoing allogeneic BMT [10]. The objectives of the present study were to characterize the pharmacokinetics of sirolimus in pediatric BMT recipients with and without concomitant use of fluconazole, and to identify factors significantly associated with variability in these pharmacokinetics.

PATIENTS AND METHODS

Study Subjects

Between September 2005 and June 2007, 4 pediatric transplant centers (Children's Hospital of Philadelphia; Methodist Children's Hospital of South Texas, San Antonio; Primary Children's Medical Center, Salt Lake City; and Children's Hospital of Pittsburgh) participated in a prospective phase II trial of sirolimus-based GVHD prophylaxis. The trial was approved by the Institutional Review Boards of the 4 institutions. Informed consent was obtained from guardians and assent or consent was obtained from patients in accordance with the Declaration of Helsinki.

Conditioning Regimen and GVHD Prophylaxis

All patients underwent BMT for high-risk acute lymphoblastic leukemia. The preparative regimen consisted of 1200 cGy fractionated total body irradiation, 10 mg/kg thiopeta, and 120 mg/kg cyclophosphamide. GVHD prophylaxis consisted of sirolimus, tacrolimus, and methotrexate. Sirolimus was given without a loading dose, at a starting dose of 2.5 mg/m²/day and with target trough levels of 3–12 ng/mL. Tacrolimus was started on day 2 as a continuous infusion at a starting dose of 0.03 mg/kg/day and with a target concentration of 5–10 ng/mL. Methotrexate was given i.v. at a dose of 5 mg/m² for 4 or 5 doses.

Blood Sampling and Analytical Assays

To characterize the pharmacokinetics of sirolimus, multiple serial blood samples (0.5–1 mL) were collected within a single oral dosing interval from each patient. Blood sampling was performed immediately before (0 hour) and at 0.5, 1, 2, 4, 6, 12, and 24 hours after administration of a minimum of 4 oral doses to allow achievement of steady state. Additional trough samples were also collected from each patient for therapeutic drug monitoring as part of clinical care at each study site. Whole-blood concentrations of

sirolimus were measured using modification of a validated HPLC/mass spectrometry (MS) method [11]. The coefficient of variation of the assay was <10% of all concentrations tested.

Noncompartmental Pharmacokinetic Analysis

The difference in trough concentrations before oral dosing (C_0) and at 24 hours after oral dosing (C_{24}) was tested using a paired 2-tailed Student t test to confirm the attainment of steady state. The area under the concentration-versus-time curve specific for the dose evaluated (AUC_{0-24}) was calculated using the trapezoid rule. Various pharmacokinetic parameters were calculated by noncompartmental analysis using Win-Nonlin version 4.1 (Pharsight, Mountain View, CA). The terminal disposition rate constant (λ_z) and terminal disposition half-life ($t_{1/2}$) were derived from data points during the terminal disposition phase, when at least 3 data points were available. Projected trough concentrations (C_{24}) were used if the observed C_{24} value was missing. Statistical comparisons of different parameters were made using a paired 2-tailed Student t test with R. A P value of <.05 was considered statistically significant.

Covariate Relationship Exploration

Covariate relationships were first visually evaluated by plotting an empirical Bayesian estimate against covariates. Covariate effects were then tested by incorporating covariates into the base model (without a covariate) one at a time using at least 13 approaches to associate the covariate with the parameter. Different cutoff values for the covariates were tested as well. A covariate was considered significant and a cutoff value was considered optimal if all of the following criteria were met: (1) a decrease in the objective function value of 6.63 for 1 degree of freedom ($P < .01$), (2) no significant trend in empirical Bayesian estimates versus covariate plots, (3) improved goodness-of-fit, (4) reduced interindividual variability, and (5) clinical plausibility of incorporating the covariate. The final model was obtained using the standard forward addition and reverse removal approach with the same criteria. The adequacy of fitting was examined by plotting predicted versus observed concentrations (goodness of fit) versus time profiles and weighted residuals versus predicted concentrations.

Population Pharmacokinetic Analysis

A nonlinear mixed-effects pharmacokinetic model (base model) was developed using NONMEM 7 (GloboMax, Hanover, MD) and a first-order conditional estimation method with interaction. Correlations among pharmacokinetic parameters were also incorporated and estimated. One-compartment and 2-compartment models were tested with first-order and zero-order elimination. Interindividual variability was described using the exponential model

$$P_{ij} = TV(P_j) \times e^{\eta^{ij}},$$

where P_{ij} is the i th individual's estimate of the j th pharmacokinetic parameter, $TV(P_j)$ is the typical value of the j th pharmacokinetic parameter, and η^{ij} is a random variable for the i th

individual and the j th pharmacokinetic parameter distributed with mean 0 and variance ω_j^2 . Various residual variability models were tested, including the following:

- Additive error model: $C_{obs} = C_{pred} + \varepsilon$
- Proportional error model: $C_{obs} = C_{pred} \times (1 + \varepsilon)$
- Combined error model: $C_{obs} = C_{pred} \times (1 + \varepsilon) + \varepsilon'$
- Exponential error model: $C_{obs} = C_{pred} \times e^\varepsilon$.

Here C_{obs} and C_{pred} are the observed and predicted concentrations, and ε and ε' are normal random variables with mean 0 and variance of δ^2 and δ'^2 , respectively. To estimate clearance normalized to body weight, an additional base model was also developed with the clearance coded as

$$CL_i = WT_i \times TV(CL) \times e^{\eta_i}$$

RESULTS

Patient and Transplant Characteristics

Pharmacokinetic profiles of sirolimus were evaluated in 40 pediatric BMT recipients. Patient and transplantation characteristics are summarized in Table 1. Sirolimus was initiated on day 0 in 38 patients, on day +1 in 1 patient, and on day +2 in 1 patient. The mean oral sirolimus dose was 2.5 ± 1.0 mg (range, 1–5 mg) once daily on the day of the study. The tacrolimus dose ranged from 0.00 to 0.066 mg/kg/day given as a continuous i.v. infusion. Blood samples for sirolimus pharmacokinetics were collected after a median of 6 doses (range, 4–10 doses). Twenty-five of 40 patients received fluconazole prophylaxis. Collectively, for these 40 patients, 232 of 259 sirolimus doses (89.6%) were the same as the first dose during the pharmacokinetic study. Thirty of 40 patients received the same initial sirolimus dose daily before pharmacokinetic samples were collected. Sirolimus doses were decreased in the other 10 patients based on clinical trough levels; all of these patients were on fluconazole. None of the 15 patients who did not receive fluconazole received another azole; 13 patients received low-dose amphotericin-B, and 2 received micafungin for anti-fungal prophylaxis.

Pharmacokinetic Analysis of Sirolimus

The observed blood concentrations of sirolimus (C_0) during the dosing interval and the mean concentrations of sirolimus in all the study subjects are shown in Figure 1. The patients were divided into 4 groups: those not receiving fluconazole, those receiving concomitant i.v. fluconazole, those receiving concomitant oral fluconazole, and those who vomited after sirolimus administration. There was a wide interindividual variation in the whole-blood sirolimus concentrations as shown in the left panel. Population mean profiles for patients are shown in the right panel. Most sirolimus whole-blood concentrations (79%) were maintained within the target range of 3–12 ng/mL, whereas 9% of sirolimus whole-blood concentrations were <3 ng/mL and 12% were >12 ng/mL.

Noncompartmental pharmacokinetic analysis was performed on 33 patients after excluding 5 patients with extremely atypical profiles who had fewer than 3 data points during the terminal disposition phase and 2 others who vomited after the sirolimus dose on the study day. As shown in Table 2, there was considerable interpatient variation in the pharmacokinetic parameters for sirolimus. The sirolimus concentrations (mean \pm SD) were as follows: trough level before the dose (C_0), 8.0 ± 4.6 ng/mL; peak concentration (C_{\max}), 19.9 ± 11.8 ng/mL; and trough level 24 hours later (C_{24}), 9.1 ± 5.3 ng/mL. The difference between the trough concentrations $(C_{24} - C_0)/C_{24}$ averaged 10.3% and was not significantly greater than 0 ($P = .75$). The C_0 and C_{24} were moderately correlated with AUC_{0-24} (0.52 and 0.51, respectively). In patients at a steady state ($n = 22$), excluding those with prolonged absorption, C_0 and C_{24} correlated well ($R^2 = 0.77$), with a slope of 0.99, indicating achievement of a steady state. The time to maximum concentration was 3.3 ± 1.6 hours, terminal disposition half-life ($t_{1/2}$) was 24.5 ± 11.2 hours, AUC_{0-24} was 401.1 ± 316.3 ng·h/mL, apparent oral clearance (Cl/F) was 0.19 ± 0.18 L/h/ kg of body weight, and apparent oral volume of distribution (Vd/F) was 5.78 ± 5.70 L/kg.

On covariate analysis, the average Cl/F of sirolimus was 3-fold greater and Vd/f was 2-fold greater in patients age ≤ 12 years than in those age >12 years. The mean sirolimus $t_{1/2}$ was 21.8 hours in patients age ≤ 12 years and 29.2 hours in those age >12 years (Figure 2). The dose-normalized sirolimus C_{24} was 1.7-fold greater and the dose-normalized AUC_{0-24} was 2-fold greater in Caucasian patients ($n = 22$) compared with Hispanic patients ($n = 9$) (Figure 3). There was no significant difference in mean age between the Caucasian and Hispanic patient groups (11.5 ± 5.2 years versus 10.2 ± 5.9 years; $P = .59$). Trough sirolimus concentrations (C_{24}) were significantly lower in patients who developed grade III–IV aGVHD ($n = 10$) than in those with grade 0–II aGVHD ($n = 22$) (6.11 ± 2.89 ng/mL versus 9.42 ± 5.52 ng/mL; $P = .044$). None of the other variables evaluated in the study (sex, body weight, hemoglobin, bilirubin, aspartate aminotransferase, alanine aminotransferase, albumin, blood urea nitrogen, and serum creatinine) were significantly associated with pharmacokinetic parameters of sirolimus (data not shown).

Effect of Concomitant Fluconazole on Pharmacokinetics of Sirolimus

Twenty-five patients received fluconazole prophylaxis at a mean dose of 201 ± 93.8 mg (5.4 ± 1.3 mg/kg) once daily, starting on day 6 (range, day 6 to day 5). Fluconazole was administered i.v. in 16 patients and orally in 6 patients. Sirolimus doses were not significantly different in pediatric BMT recipients with concomitant fluconazole and those without concomitant fluconazole (Table 2). Sirolimus trough concentrations were significantly higher in patients receiving fluconazole compared with those not receiving fluconazole (Figure 4). Dose-normalized sirolimus C_0 values were 3.93 ± 2.5 ng/mL/mg in patients receiving concomitant fluconazole versus 2.35 ± 1.5 ng/mL/mg in those not receiving concomitant fluconazole ($P = .0299$), and corresponding C_{24} values were 4.8 ± 3.34 ng/mL/mg versus 2.5 ± 1.7 ng/mL/mg ($P = .0177$).

The population pharmacokinetic analysis included a total of 333 sirolimus concentrations from 37 patients. Two patients who vomited and 1 patient with extreme atypical pharmacokinetic profiles were excluded, because it was not possible to calculate any

pharmacokinetic parameters from these patients. A 2-compartment model with first-order absorption and elimination adequately described the data. The population pharmacokinetic estimates are shown in Table 3. These estimates are consistent with the results from the noncompartmental analysis. Interindividual variability was estimated for clearance (78%); volume of distribution of central compartment, V_c (91%); and absorption rate constant, k_a (63%). The residual variability was best described using the following combined error model:

$$C_{obs} = C_{pred} \times (1 + \varepsilon) + \varepsilon'$$

The proportional and additive residual variabilities were 0.21 and 0.84 ng/mL, respectively. The additive error estimate was lower than the lowest limit of quantification of the assay (2 ng/mL). Individual predictions agreed well with our observations. Weighted residuals were approximately normally distributed. None of the variables evaluated in this study was significantly associated with any sirolimus pharmacokinetic parameters.

DISCUSSION

Previous studies in BMT recipients have examined relationship of sirolimus dose and drug levels with clinical outcomes such as microangiopathy, sinusoidal obstruction syndrome, GVHD and survival [1,2,12,13]. Although the pharmacokinetics of sirolimus have been well characterized in adult and pediatric organ transplant recipients [9,14,15], to our knowledge this is the first systematic study of sirolimus pharmacokinetics in a BMT population. Here we report the results of sirolimus pharmacokinetics in pediatric patients treated uniformly on a common total body irradiation-based conditioning regimen and sirolimus and tacrolimus-based GVHD prophylaxis.

The mean AUC_{0-24} of sirolimus in this study was 401.1 ± 316.3 ng·h/mL, nearly double the values previously reported in adult kidney transplant recipients (173 ± 50 ng·h/mL), pediatric liver transplant recipients (168.3 ± 86.5 ng·h/mL), and pediatric small bowel transplant recipients (177.4 ± 72.1 ng·h/mL) [9,14]. More important, the corresponding Cl/F of sirolimus was one-half the value reported in pediatric liver and small intestinal transplant recipients. The Cl/F in our patients appears to be closer to the Cl/F reported in pediatric kidney transplant recipients on calcineurin inhibitor therapy [15]. The lower Cl/F of sirolimus in our patient population may reflect the functional status of the liver (ie, less ability to metabolize the drug) or greater bioavailability of the drug (owing to decreased presystemic metabolism) in this group of patients. Given the lack of an i.v. formulation of sirolimus at this point, it is not possible to distinguish between these 2 factors. Of note, the pharmacokinetic study was performed in these patients early in the course of a myeloablative regimen, when the effects of preparative regimens on liver and intestine are significant. It is possible that with time and after a conditioning regimen, the Cl/F may increase and the AUC_{0-24} may decrease in these patients relative to values observed in solid organ transplant recipients. Another reason for the lower Cl/F in our population may be related to the routine use of steroids in solid organ transplant recipients, leading to increased

sirolimus metabolism and thus a lower AUC_{0-24} in solid organ transplant recipients compared with BMT recipients.

Trough blood concentrations of sirolimus were closely correlated with AUC_{0-24} values in our pediatric BMT recipients ($R^2 = 0.52$ for all patients and $R^2 = 0.77$ when patients with prolonged absorption were excluded). This finding is consistent with the good correlations reported in pediatric liver and intestinal transplant recipients ($R^2 = 0.85$) [9], pediatric kidney transplant recipients on a calcineurin-free protocol ($R^2 = 0.84$) [8], and pediatric kidney transplant recipients on tacrolimus cotherapy ($R^2 = 0.68$) [15].

In the present study, mean sirolimus $t_{1/2}$ was 24.5 ± 11.2 hours, similar to the half-life reported in pediatric liver transplant recipients (21.2 ± 14.1 hours) and small bowel transplant recipients (19.3 ± 5.6 hours) who received sirolimus and tacrolimus immunosuppression [9]. Shorter $t_{1/2}$ (9.7 hours at 1 month posttransplantation and 10.8 hours at 3 months posttransplantation) have been reported in pediatric renal transplant patients treated on an every-12-hour schedule on a calcineurin inhibitor-free protocol [8]. In contrast, longer $t_{1/2}$ (mean values of 47–107 hours) after a single dose of sirolimus have been reported in pediatric patients with stable chronic renal failure undergoing dialysis [16]. These long half-lives are comparable to the 57–63 hours reported in adult kidney transplant recipients [14] and healthy volunteers after a single dose of sirolimus. The shorter half-life of sirolimus observed in our patients is consistent with the shorter half-lives of other drugs, such as cyclosporine and tacrolimus, in pediatric transplant recipients compared with adult transplant recipients.

Age has been shown to impact the $t_{1/2}$ and Cl/F of tacrolimus and sirolimus. In this study, although the mean $t_{1/2}$ was 24 hours, the value was lower in patients age ≤ 12 years compared with those age >12 years. The weight-normalized Cl/F was also 3-fold greater in patients age ≤ 12 years compared with those age >12 years. Schachter et al. [8] reported a significantly shorter terminal $t_{1/2}$ in the younger age group (≤ 6 years: 8.2 hours; range, 4.4–10.6 hours; >6 years: 12.6 hours, range, 4.7–95.2 hours; $P < .05$) in kidney transplant recipients on a calcineurin inhibitor-free protocol. The same group reported a higher apparent clearance of sirolimus in patients age 0–5 years with or without concomitant calcineurin inhibitors [15]. Age-dependent changes in the expression and activity of cytochrome CYP3A isoenzymes and P-gp may contribute to the observed variation in sirolimus clearance. Because sirolimus is not available in an i.v. formulation, it is not possible to estimate the contribution of differences in clearance and the bioavailability of sirolimus to the observed changes in sirolimus exposure in older and younger pediatric patients. The mean $t_{1/2}$ values seen in the present study support the practice of once-daily dosing of sirolimus in pediatric BMT recipients in general. Patients with shorter half-lives may require higher doses or every-12-hour dosing of sirolimus to reach target sirolimus trough concentrations. If the measured trough blood concentration is below but closer to the target, then a simple increase in dose may be appropriate; in cases where the trough concentration is farther below the target, a dosage increase and every-12-hour dosing may be more logical.

African-American renal allograft recipients have poorer renal allograft survival and higher mortality compared with Caucasian recipients [17]. Differences in bioavailability and the systemic exposure of calcineurin inhibitors and mammalian target of rapamycin inhibitors (sirolimus and everolimus) may be key contributing factors to the observed differences in posttransplantation outcomes. African Americans are known to have a higher Cl/F and lower oral bioavailability of sirolimus compared with Caucasians. Hispanics also have been reported to have a lower bioavailability of tacrolimus compared with Caucasians [18]. No differences in pharmacokinetics for other drugs, including nortriptyline, have been reported between Hispanics and Caucasians [19]. In the present study, the dose-normalized steady-state C_{24} concentration of sirolimus was 1.7-fold greater ($P = .036$) and the dose-normalized AUC_{0-24} was 2-fold greater ($P = .012$) in Caucasian patients ($n = 22$) compared with Hispanic patients ($n = 9$). It is possible that racial/ethnic variations in the pharmacokinetics of drugs may depend on the enzyme studied and the substrate used. Racial/ethnic differences may be related to various genetic and nongenetic factors, including known genetic variations that influence transporter/enzyme activity such genes as *CYP3A4*, *CYP3A5*, and *ABCB1* *MDR1* [3]. Sirolimus is a substrate for cytochrome P450 3A (3A4 and 3A5) and P-gp enzymes in the gut and liver. The proportions of *CYP3A5* functional alleles were significantly higher in African Americans (81.4%) and Hispanics (43.1%) compared with Caucasians (16.8%) [20]. Our study was not designed to address whether the observed differences are related to the frequency of *CYP3A5* alleles among different racial/ethnic groups. These findings of lower steady-state C_{24} and AUC_{0-24} values in Hispanic patients require validation in larger independent cohorts.

In this study, the cumulative incidence of grade II–IV and grade III–IV aGVHD at 180 days was 38% and 21%, respectively [10]. The mean sirolimus C_{24} value was significantly lower in patients who developed grade III–IV aGVHD ($n = 10$) compared with those with grade 0–II aGVHD ($n = 22$) (6.11 ± 2.89 ng/mL versus 9.42 ± 5.52 ng/mL; $P = .044$). Malard et al. [21] reported a significant association between low cyclosporine levels in the first 2 weeks posttransplantation and an increased risk of grade III–IV aGVHD. Jacobson et al. [22] reported a direct correlation between grade III–IV aGVHD and tacrolimus clearance. It is not possible to distinguish whether the low concentrations were related to lower bioavailability because of GVHD or whether the lower concentrations in these patients predisposed them to higher-grade GVHD. Moreover, these “low” sirolimus levels are within the study’s target range, and given the lack of serial samples and data at the onset of GVHD, this finding should be considered preliminary and needs to be tested in a larger independent cohort before generalizable conclusions can be drawn.

As reported previously, 7 of the 63 subjects in the clinical study developed veno-occlusive disease of liver and 3 developed thrombotic microangiopathy [10]. Given the concerns about these toxicities, particularly when sirolimus and tacrolimus are used together, it would be of interest to examine the relationship between sirolimus exposure and these outcomes. The present single-point pharmacokinetic study did not collect sufficient clinical drug level data on all patients to allow such a correlative analysis.

Sirolimus trough concentrations were significantly higher in patients on concomitant fluconazole than in those not on concomitant fluconazole (C_0 : 3.93 ± 2.5 versus 2.35 ± 1.47

ng/mL/mg [$P = .030$]; C_{24} : 4.8 ± 3.34 versus 2.51 ± 1.72 ng/mL/mg [$P = .018$]). Sirolimus is metabolized extensively by the CYP3A system in the liver and is a substrate of the P-gp transporter system. Fluconazole is an inhibitor of CYP3A4 and P-gp, although it is the weakest in vitro inhibitor of CYP3A4 compared with itraconazole, voriconazole, and posaconazole [7,23]. More potent interactions have been described with extended-spectrum azoles, voriconazole, and posaconazole, and more drastic sirolimus dosage reductions have been recommended [6,24]. The general recommendation is to reduce the sirolimus dose by nearly 33% when concomitant fluconazole treatment is necessary. To date, there have only been 2 case reports of interaction between sirolimus and fluconazole [5,25]. Our finding of 1.7- to 1.9-fold greater sirolimus concentrations with the concomitant use of fluconazole is consistent with the predicted inhibition of sirolimus metabolism and underscores the importance of closely monitoring sirolimus blood concentrations. Before the pharmacokinetic blood draws, 10 of 20 patients on fluconazole therapy had their sirolimus dose decreased based on sirolimus therapeutic drug monitoring, compared with none of the patients without fluconazole therapy. This dose decrease introduced bias; the observed impact of fluconazole in this study might have been greater had no dosage adjustments been made before the formal pharmacokinetic analysis.

In conclusion, our findings in this study of pediatric BMT recipients can be summarized as follows: (1) significant interindividual variability in the exposure of sirolimus and in its pharmacokinetics; (2) reasonably good correlation between trough sirolimus blood concentrations and drug exposure (AUC_{0-24}); (3) lower apparent clearance of sirolimus compared with that in solid organ transplantation recipients, at least in the early post-BMT period; (4) higher apparent clearance in younger patients than in older pediatric patients; (5) higher blood concentrations of sirolimus when administered concomitantly with fluconazole; (6) lower drug exposure in Hispanics than in Caucasians; and (7) an inverse association between GVHD and trough sirolimus concentrations. With therapeutic monitoring, a majority (79%) of the patients receiving sirolimus were maintained within the target therapeutic range (3–12 ng/mL). This study provides a rationale and support for sirolimus dose adjustments based on steady-state blood concentrations to achieve the target concentration to minimize toxicity and maximize therapeutic benefits in pediatric BMT recipients.

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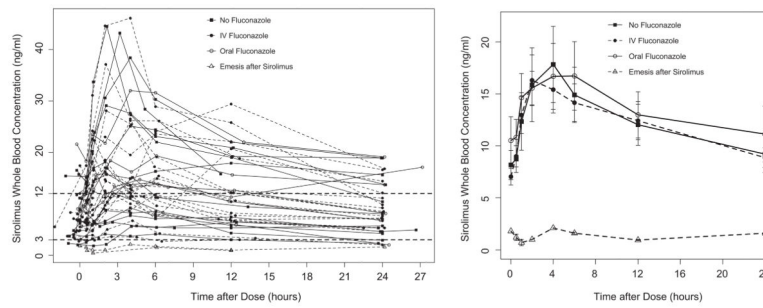


Figure 1. Whole-blood 0 h concentrations of sirolimus for all subjects ($n = 40$) over a dosing interval, individually for each patient (left) and in 4 groups (right): no fluconazole, concomitant i.v. fluconazole, concomitant oral fluconazole, and vomiting after sirolimus administration.

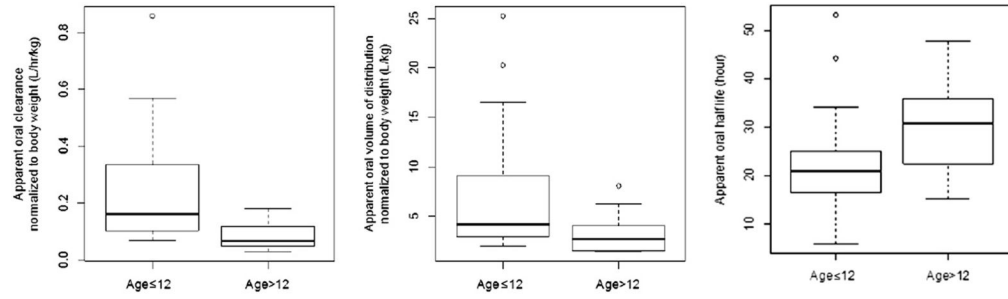


Figure 2. Standard box-and-whisker plots showing Cl/F, Vd/F, and $t_{1/2}$ of sirolimus in the ≤ 12-year and >12-year age groups. Solid horizontal lines represent median values.

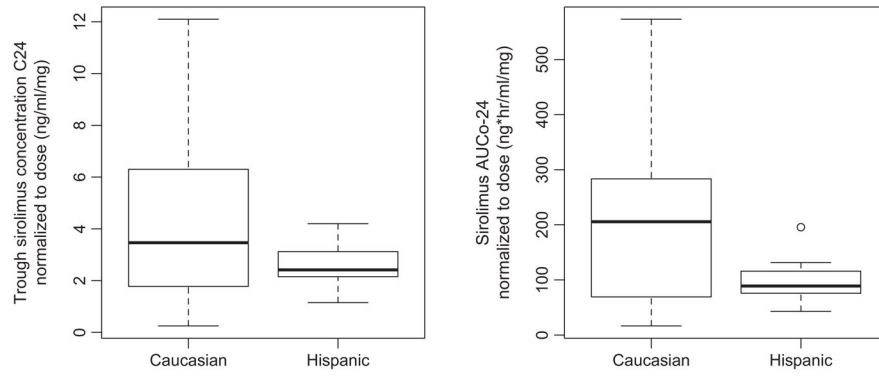


Figure 3. Standard box-and-whisker plots showing sirolimus trough concentrations and AUC₀₋₂₄ in Caucasian and Hispanic patients. Solid horizontal lines represent median values.

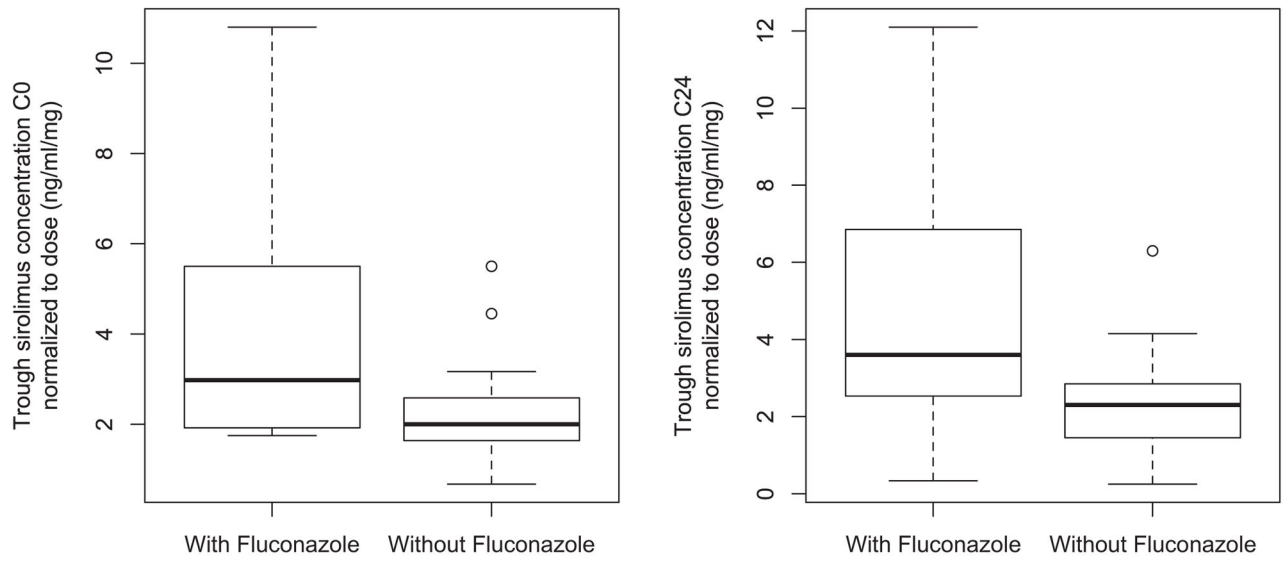


Figure 4. Standard box-and-whisker plots showing dose-normalized trough sirolimus concentrations (C_0 and C_{24}) in patients receiving concomitant fluconazole therapy and those not receiving fluconazole. Values are median and 95% CI. Solid horizontal lines represent median values.

Table 1

Patient and Transplant Characteristics

Characteristic	Value
Number of patients	40
Age, years, median \pm SD (range)	10.1 \pm 5 (4–22)
Weight, kg, median \pm SD (range)	34.8 \pm 19.0 (13.2–84.3)
Sex, n (%)	
Male	27 (67)
Female	13 (33)
Race/ethnicity, n (%)	
Caucasian	27 (67)
Hispanic	11 (28)
African American	1 (2.5)
Asian	1 (2.5)
Transplant type, n (%)	
Related donor	16 (40)
Unrelated donor	24 (60)
Type of donor graft, n (%) [*]	
Bone marrow	18 (45)
Umbilical cord blood	23 (58)
Peripheral blood	1 (2)

* Two patients received both bone marrow and cord blood.

Table 2

Noncompartmental Analysis of Sirolimus

	All (n = 33)	No Fluconazole (n = 11)	i.v. Fluconazole (n = 16)	Oral Fluconazole (n = 6)
Half-life, h	24.5 ± 11.2 (5.8~53.2)	26.6 ± 14.6 (5.8~53.2)	23.7 ± 9.2 (9.9~44.3)	22.8 ± 10.0 (8.6~39.0)
T _{max} , h	3.3 ± 1.6 (1.0~6.3)	3.2 ± 1.0 (2.0~4.2)	3.4 ± 1.9 (1.0~6.3)	3.4 ± 1.9 (1.0~6.1)
C _{max} , ng/mL	19.9 ± 11.8 (3.9~46.1)	21.3 ± 15.4 (4.1~44.5)	19.4 ± 11.0 (3.9~46.1)	18.7 ± 7.0 (12.1~32.0)
Cl/F, L/h	5.8 ± 4.5 (1.6~22.7)	5.7 ± 3.0 (2.2~11.1)	5.7 ± 5.2 (1.6~22.7)	6.3 ± 5.7 (1.6~17.2)
Cl/F, L/h/kg	0.19 ± 0.18 (0.03~0.86)	0.19 ± 0.13 (0.03~0.45)	0.19 ± 0.22 (0.03~0.86)	0.19 ± 0.19 (0.05~0.57)
Vd/F, L	184.6 ± 128.1 (38.6~534.7)	201.3 ± 121.3 (39.7~460.1)	182.7 ± 151.7 (38.6~534.7)	159.2 ± 71.6 (57.7~249.1)
Vd/F, L/kg	5.78 ± 5.70 (1.44~25.23)	6.64 ± 5.31 (1.48~16.56)	5.75 ± 6.95 (1.44~25.23)	4.31 ± 1.66 (2.61~7.03)
AUC, ng-h/mL	401.1 ± 316.3 (20.7~1332.3)	450.4 ± 367.2 (84.5~1332.3)	398.9 ± 236.9 (24.8~762.4)	323.5 ± 413.5 (20.7~1146.0)
AUC, ng-h/mL/mg	170.7 ± 134.7 (10.4~573)	147.4 ± 88.8 (52.9~333.1)	203.2 ± 122.9 (16.5~392.4)	139.3 ± 214.8 (10.4~573)
C ₀ , ng/mL	8.0 ± 4.6 (1.8~21.6)	6.8 ± 4.8 (1.8~16.5)	8.0 ± 4.1 (3.7~21.6)	11.0 ± 5.3 (5.6~17.4)
C ₀ , ng/mL/mg	3.4 ± 2.3 (0.7~10.8)	2.3 ± 1.5 (0.7~5.5)	4.0 ± 2.6 (1.8~10.8)	3.7 ± 2.4 (1.8~7.8)
C ₂₄ , ng/mL	9.1 ± 5.3 (1.0~19.1)	8.0 ± 6.3 (1.0~18.9)	10.0 ± 4.2 (4.6~19.1)	8.6 ± 6.5 (1.0~16.8)
C ₂₄ , ng/mL/mg	4.1 ± 3.1 (0.2~12.1)	2.5 ± 1.7 (0.2~6.3)	5.3 ± 3.5 (1.5~12.1)	3.2 ± 2.6 (0.3~6.8)
Fluconazole dose, mg*	201.0 ± 93.8 (100~400)		188.9 ± 78.4 (100~400)	237.5 ± 132.0 (100~400)
Sirolimus dose, mg*	2.5 ± 1.0 (1~5)	2.8 ± 1.3 (1~5)	2.2 ± 0.8 (1~4)	2.8 ± 1.0 (2~4)
Sirolimus formulation, n [†]	24/7/7/2	9/2/4/0	10/5/3/1	5/0/0/1

T_{max} indicates time to reach maximum concentration; C_{max}, maximum concentration; Vd/F, apparent oral volume of distribution; Cl/F, apparent oral clearance; AUC, Area under the concentration-vs-time curve specific for the dose evaluated; C₀, trough concentration prior to oral dosing; C₂₄, trough concentration at 24 hours after oral dosing.

Values are expressed as mean ± SD (range) unless indicated otherwise.

* Fluconazole or sirolimus dose administered on the day of the sirolimus pharmacokinetic study.

[†] Values are number of patients for tablet/suspension/both/unknown.

Table 3

Population Pharmacokinetic Analysis

Variable	Population Estimate (\pmStandard Error)
Clearance (Cl/F)	6.66 ± 1.10 L/h or 0.17 ± 0.03 L/h/kg
Volume of distribution of central compartment (Vc)	26.9 ± 7.7 L
Volume of distribution of peripheral compartment (Vp)	630 ± 171 L
Intercompartment clearance (Q)	4.62 ± 2.00 L/h
Absorption rate constant (ka)	0.0535 ± 0.0104 h ⁻¹