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PHARMACODYNAMICS

Repeat-dose sirolimus pharmacokinetics and pharmacodynamics in patients with hepatic allografts

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Abstract
Purpose To determine sirolimus steady-state pharmacokinetics, and to assess the relationship between time-normalized trough sirolimus concentration ($C_{min,TN}$) and evidence of efficacy (rejection and death) and adverse reactions (stomatitis and pneumonia) in liver allograft patients.
Methods Dense sampling of sirolimus was performed over a single daily-dosing interval in 11 hepatic allograft recipients on day 28 and at 3 months after start of treatment. Serial trough concentration sampling was performed in 380 hepatic allograft recipients on days 1, 7, 14, 28, 42, 60, 90, 180, 270 and 360 after start of treatment. Occurrence of stomatitis, pneumonia, rejection, and death were collected for 360 days after start of treatment. Noncompartmental pharmacokinetic parameters were analyzed in the 11 densely sampled patients; $C_{min,TN}$ was determined in the 380 patients.
Results Mean maximum concentration (C_{max}), time to C_{max} (t_{max}), area under the curve for the given dose interval (AUC_{tau}), and whole blood oral clearance at 3 months were 20.8±7.6 ng/mL, 3±1 h, 338±144 ng·h/mL, and 10.0±5.6 L/hr, respectively. In the 11 densely sampled patients, linear regression showed that $C_{min,TN}$ was highly predictive of AUC_{tau} (r^2=0.77, P<0.0001) at each analysis time point. Logistic regression showed a relationship between $C_{min,TN}$ in the 380 patients and pneumonia occurrence, but not between $C_{min,TN}$ and stomatitis, rejection, or death.
Conclusions In this study, the pharmacokinetic profile of sirolimus in hepatic allograft patients was consistent with that of renal transplantation recipients. With the exception of pneumonia, no correlation was observed between $C_{min,TN}$ and the occurrence of adverse events of interest.

Keywords Hepatic allograft · Hepatic insufficiency · Pharmacokinetics · Pharmacodynamics · Sirolimus

Abbreviations

ANOVA	Analysis of variance
AUC	Area under the concentration–time curve
AUC_{tau}	Area under the curve for the given dose interval
CI	Confidence interval
C_{max}	Maximum concentration
$C_{min,TN}$	Time-normalized trough concentration
CNI	Calcineurin inhibitor
OR	Odds ratio
t_{max}	Time to maximum concentration

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Introduction

Sirolimus, marketed as Rapamune (Pfizer Inc, Philadelphia, PA, USA), is an immunosuppressive macrocyclic lactone approved for the prophylaxis of renal allograft rejection [1]. In vitro studies show that sirolimus causes immunosuppression by inhibiting T-cell activation through the suppression of interleukin-2- and interleukin-4–driven T-cell proliferation [2, 3].

The pharmacokinetics of sirolimus have been well described [4–8]. After oral administration, sirolimus is rapidly absorbed (t_{max}, 0.67 to 3 h) and has a low clearance (0.147 to 0.208 L/h/kg; 10.3 to 14.6 L/h, assuming weight of 70 kg), a large apparent volume of distribution (8.3 to 12 L/kg), and a long terminal half-life (57 to 63 h) [9, 10]. The bioavailability of sirolimus in stable renal transplantation patients is 14%; this low bioavailability and extensive metabolism can cause significant variability.

Pharmacodynamic data are also available for sirolimus. To avoid rejection and graft loss, adequate therapeutic concentrations are required, but not so high as to worsen potential adverse reactions, including thrombocytopenia, leukopenia, or hypertriglyceridemia, as well as infections due to immune suppression [11, 12]. In a 4-year study of 150 renal transplantation patients, serial sirolimus concentrations were measured to determine potential correlates between sirolimus trough levels and clinical events. Trough concentrations exceeding 15 ng/mL were determined to increase the incidence of hypertriglyceridemia and thrombocytopenia [12]. Another study showed that trough concentrations >11 ng/mL were found to correlate with the occurrence of hypertriglyceridemia, of 14 ng/mL with thrombocytopenia, and of >15 ng/mL with leukopenia, with levels <5 ng/mL associated with the occurrence and severity of acute rejection episodes [11]. Similarly, a review of sirolimus clinical trials revealed that concentrations >6 ng/mL were necessary for full immunosuppressive effect (when administered in combination with cyclosporine and corticosteroids). These sirolimus therapeutic windows were developed in patients at standard risk of rejection; in patients who received cyclosporine- or corticosteroid-sparing regimens or who were considered high-risk patients, higher concentrations were potentially needed to reach an appropriate immunosuppressive response, highlighting the significance of intrapatient variability that exists among potential recipients of sirolimus treatment [11, 12].

Because the pharmacokinetics of sirolimus vary between individuals and because there is a defined exposure associated with good efficacy while minimizing toxicity, therapeutic drug monitoring is performed frequently in patients who have received renal allografts. A relationship has been shown between the steady-state area under the concentration–time curve (AUC_{tau}) and steady-state trough concentrations [9]. Consequently, determining whether a similar relationship exists for patients with hepatic allografts would serve as a valuable clinical resource in this patient population.

Pharmacokinetic studies of sirolimus have not been performed in patients receiving hepatic allografts. Dosing of postoperative drug therapy is greatly influenced by the drug-metabolizing capacity of the donor liver. Age and factors involved in the process of liver transplantation (e.g., organ preservation, reperfusion injury, inflammatory changes, and the immunological response of the recipient) can affect drug metabolic function [13]. Further, drug metabolism appears to be most compromised immediately after hepatic transplantation. A population pharmacokinetic study in patients undergoing hepatic transplantation showed that tacrolimus clearance had stabilized by 15 days' post-transplantation [14]. Longer-term changes in metabolism have also been shown using intravenous and oral midazolam (CYP3A4 substrate) dosing. A case report showed that the clearance of midazolam increased and bioavailability decreased by approximately 50% from 12 to 27 months post-transplantation in a hepatic transplantation patient, suggesting altered first-pass effects [15].

When considering the narrow therapeutic range and variable pharmacokinetics of sirolimus, as well as the effect of hepatic impairment on sirolimus pharmacokinetics and the plausible change in metabolic function in liver transplantation patients, it is important to have an understanding of sirolimus pharmacokinetics in this patient population. The present study aimed first to determine the pharmacokinetics of sirolimus in patients with liver allografts, and second, to assess the relationship between time-normalized trough sirolimus concentration ($C_{min,TN}$) and evidence of efficacy (rejection and death) and adverse reactions (stomatitis and pneumonia) in patients with liver allografts.

Materials and methods

Patients

This pharmacokinetic–pharmacodynamic study was part of a larger randomized, open-label, parallel-group, comparative outpatient study evaluating the impact on renal function of the conversion from a calcineurin inhibitor (CNI), i.e., tacrolimus or cyclosporine, to sirolimus-based maintenance immunosuppressive therapy compared with the continued use of CNIs in hepatic transplantation recipients [16].

Patients were eligible if they had received orthotopic hepatic transplantation within 6 to 144 months of randomization and were receiving immunosuppressive therapy with a stable regimen of CNI or a combination of CNI with or without antimetabolite therapy (azathioprine or mycophenolate mofetil) for a minimum of 4 weeks prior to randomization. Patients were required to have normal white blood cell and platelet counts, be at least 13 years old, weigh at least 40 kg, and have Cockcroft–Gault glomerular filtration rate values between ≥40 mL/min and ≤90 mL/min. Additional eligibility criteria included normal triglyceride and cholesterol levels, as well as no evidence of thrombosis or stenosis of the hepatic artery, hepatic vein, or portal vein.

If patients were hepatitis C-negative, hepatic transaminases <3 times the upper limit of normal on 2 consecutive determinations within 3 months of assignment were required. Hepatitis C-positive patients were required to have hepatic transaminases <5 times the upper limit of normal on 2 consecutive determinations within the 3 months before assignment. Patients with disorders potentially affecting pharmacokinetic assessment, hypersensitivity to sirolimus, or acute disease, or those taking other investigational drugs were excluded from the study.

The present multicenter study was conducted in accordance with the Declaration of Helsinki, its amendments, and local laws and guidelines, and was approved by the appropriate institutional committee at each site. Written informed consent was obtained from all patients prior to study participation.

Study design

Patients enrolled in the study were randomized 2:1, either switching to sirolimus or continuing on CNI-containing immunosuppression. Patients receiving cyclosporine in the 4 weeks before randomization were required to continue cyclosporine treatment for 90 days following the first dose of sirolimus. After 90 days, the cyclosporine was eliminated, restarted, or discontinued, at the investigator's discretion. Patients were permitted to receive antimetabolite therapy agents (e.g., azathioprine, mycophenolate mofetil) if required.

Patients randomized to sirolimus received a loading dose of sirolimus of 10 to 15 mg in divided doses on day 1; on days 2 through 6, a sirolimus dose of 3 to 5 mg/day was administered. For the remaining study period, sirolimus doses were titrated to attain the protocol-specified trough concentrations of 8 to 16 ng/mL (using a chromatographic method) or 10 to 20 ng/mL (using an immunoassay).

On day 28 and at the month 3 visit, dense pharmacokinetic sampling was performed in selected patients by collecting venous whole blood (3 mL) at the following time points: predose and 1, 2, 3, 4, 6, 12, and 24 h postdose. All blood samples were collected in tubes containing solubilized ethylenediaminetetraacetic acid. The entire contents of the sample were then transferred to another tube that was stored at approximately 4°C for up to 48 h or frozen at a temperature of at least −20°C when shipment occurred more than 48 h after sampling.

All patients participating in the trial were monitored regularly. Sirolimus trough concentrations were determined throughout the study, minimally at days 1, 7, 14, 28, 42, 60, 90, 180, 270, and 360 after start of treatment. Concentrations were also determined after dose adjustments and, whenever possible, at the time of any drug-related adverse event or suspected acute rejection. Cases of stomatitis, pneumonia, rejection, and death were recorded for all patients.

Bioanalysis

Whole blood samples collected from the dense sampling were analyzed for sirolimus using a microparticle immunoassay. Briefly, the extraction step involved a mixture of 150 μL whole blood ethylenediaminetetraacetic acid sample with 300 μL of a precipitating reagent. This mixture was then vortexed and centrifuged, following which the supernatant was added to the sample well of the IMx® disposable reaction cell (Abbott Laboratories, Quebec, Canada). The marker was sirolimus-alkaline phosphatase conjugate and the substrate 4-methylumbelliferyl phosphate. The limit of quantitation was approximately 1.3 ng/mL, with a coefficient of variation of 20% [17, 18]. Whole blood samples collected for therapeutic drug monitoring were assayed at each investigator's local laboratory, and the results were recorded. Assay results from immunoassays were converted to high-performance liquid chromatography (HPLC) equivalents prior to pharmacokinetic analysis.

Pharmacokinetic analysis

The pharmacokinetic parameters were determined for the patients undergoing dense sample collection via a noncompartmental approach using WinNonlin Pro version 5.1.1 (Pharsight Corp, Cary, NC, USA). The maximum concentration (C_{max}) and the time to reach C_{max} (t_{max}) were obtained directly from the concentration–time plots. Sirolimus whole blood area under the curve for the given dose interval (AUC_{tau}) was calculated using the log-linear trapezoidal method with a weighting scheme of 1/Y. Apparent clearance was calculated as a ratio of dose to AUC_{tau}.

Time-normalized trough concentrations ($C_{min,TN}$) were determined for sirolimus in each individual using the area method for the interval 1 to 52 weeks, calculated as $C_{min,TN} = AUC_{i\text{-}j}/tj\text{-}ti$, where AUC is the area under the concentration–time curve, "i" is the beginning of the interval, and "j" is the end of the interval. $C_{min,TN}$ were also determined in a similar manner up to the time of an event of interest in individuals who had evidence of lack of efficacy (rejection, death) or toxicity (stomatitis or pneumonia).

Statistical analysis

Summary statistics were calculated for the pharmacokinetic parameters during both dense sampling (C_{max}, T_{max}, AUC_{tau}, apparent clearance) and therapeutic drug monitoring ($C_{min,TN}$). A simple linear regression procedure was performed using the trough concentration (C24), measured

on days 28 and 90, as the explanatory variable and AUC_{tau} as the response variable.

The influence of sirolimus $C_{min,TN}$ on events of interest (i.e., pneumonia, stomatitis, rejection episodes, and death) was examined. The $C_{min,TN}$ values that were determined for each patient experiencing an adverse event of interest at the time closest to the first occurrence were compared with $C_{min,TN}$ calculated over the interval of 1 to 52 weeks for patients who did not experience the events of interest using analysis of variance methods. Logistic regression analysis was used to test whether $C_{min,TN}$ contributed to the risk of occurrence of the events of interest.

Results

Patients

In total, 11 patients participated in the dense sampling group, of whom 6 were tested on both day 28 and at the 3-month visit. Individual demographic data for the 11 densely sampled patients are shown in Table 1.

Sirolimus trough concentrations were measured in 380 out of 393 patients randomized to sirolimus. The following observations were made in the patients randomized to sirolimus. The study population consisted of male (69%) and female patients (31%) aged 21 to 76 years, with a mean age of 55.4 years. The most common causes of hepatic failure were alcoholic liver disease (33%), hepatitis B (20%), and hepatitis C (17%). All patients were between 6 and 144 months' post-transplantation, with the following distribution of time: <12 months, 12.2%; 12 to <39 months, 37.2%; 39 to <60 months, 20.4%; and >60 months, 30.3%. Mean baseline AST was 27.5 (±16.7) mU/mL, and mean ALT was 30.4 (±26.1) mU/mL.

Pharmacokinetics

Mean sirolimus concentration–time profiles on day 28 and at 3 months are shown in Fig. 1, with pharmacokinetic parameters shown in Table 2. No difference was observed between the mean values for daily dose, C_{max}, and AUC on the two occasions when all patients were considered; however, the apparent clearance decreased in 5 of 6 patients studied at both time points.

Figure 2 shows the sirolimus trough concentrations for the duration of the study. As expected with sirolimus pharmacokinetics, considerable variability was noted in the trough concentrations. Overall, however, the mean trough concentrations, because of the defined target range, were consistent over time. Sirolimus $C_{min,TN}$ (mean ± standard deviation) over the first year was 12.6±4.4 ng/mL for the 270 patients for whom data were available. Regression of C24 on AUC_{tau}, as depicted in Fig. 3, showed a significant relationship: $AUC_{tau}=80.8*C24+19.3$ ($r^2=0.797$, $P<0.0001$).

Pharmacodynamics

The $C_{min,TN}$ results for the 177 sirolimus-treated patients who developed stomatitis and for the 212 patients who did not experience stomatitis are shown in Table 3. The analysis of variance (ANOVA) failed to detect a difference in $C_{min,TN}$ between these two sets of patients. The logistic regression failed to show $C_{min,TN}$ as being a significant factor for stomatitis (odds ratio [OR]=0.999; 95% confidence interval [CI], 0.950–1.051).

The $C_{min,TN}$ results for the 13 sirolimus-treated patients who developed pneumonia and for the 376 patients who did not experience pneumonia are shown in Table 3. The ANOVA showed that $C_{min,TN}$ in those who developed

Table 1 Baseline demography of densely sampled patients

Patient	Age	Sex	Ethnic origin	Weight, kg
1	55	Male	White	83.7
2	51	Male	White	92.8
3	55	Male	White	72.4
4	28	Female	White	71.0
5	64	Male	White	64.0
6	60	Male	White	87.0
7	64	Female	White	104
8	51	Male	White	90.0
9[a]	60	Female	White	74.3
10	61	Male	White	76.0
11	53	Female	White	69.0
Mean ± SD	55±10	36% female	100% white	79.7±12.5
Mean ± SD (study as a whole)	55±10	31% female	80% white	78.7±17.8

[a]Patient had history of hepatitis C

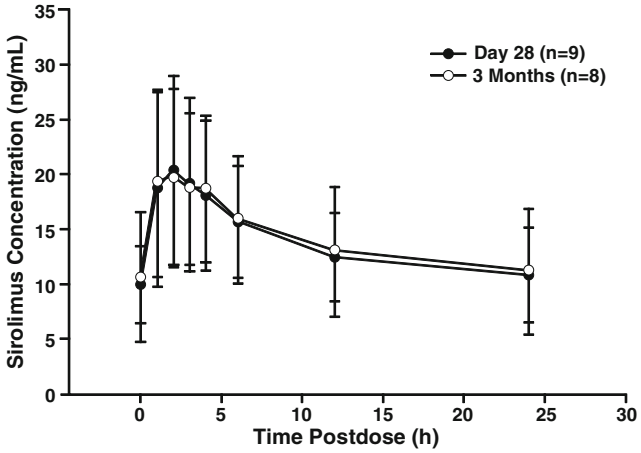

Fig. 1 Mean ± SD steady-state sirolimus concentrations on day 28 and at 3 months in patients with hepatic allografts. *Closed circles* represent day-28 (n=9) and *open circles* represent 3-month results (n=8)

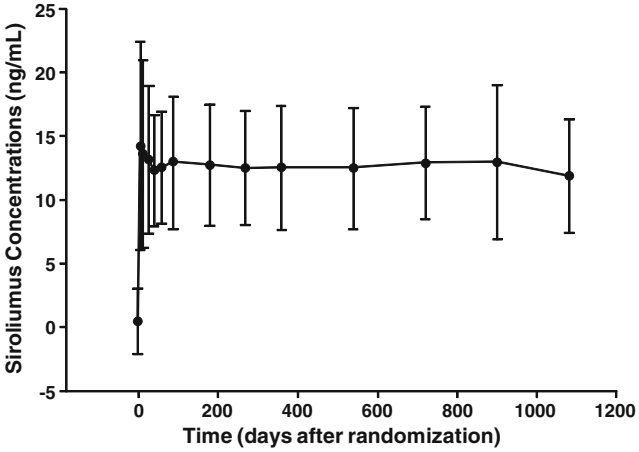

Fig. 2 Mean ± SD sirolimus trough concentrations in patients with hepatic allografts (n=380)

pneumonia was higher than in those who did not. The logistic regression also showed $C_{min,TN}$ as being a significant factor for developing pneumonia (OR=1.167; 95% CI, 1.016–1.340).

The $C_{min,TN}$ results for the 41 sirolimus-treated patients who experienced and the 348 patients who did not experience a rejection episode are shown in Table 3. The ANOVA failed to detect a difference in $C_{min,TN}$ in those who had a rejection and those who did not. The logistic regression failed to show $C_{min,TN}$ as being a significant factor for rejection episodes (OR=0.974; 95% CI, 0.871–1.091).

The $C_{min,TN}$ results for the 9 sirolimus-treated patients who died vs the 380 patients who did not die are shown in Table 3. The ANOVA failed to detect a difference in $C_{min,TN}$ between these two sets of patients. The logistic regression

Table 2 Steady-state sirolimus pharmacokinetics in patients with hepatic allografts

Patient	Dose (mg/day)	C_{max} (ng/mL)	t_{max} (h)	AUC_{tau} (ng·h/mL)	CL/F (L/h)
Day 28					
1	3	16.9	1	234	12.8
2	1	7.9	6	288	6.85
3	3	32.6	3	528	5.68
4	5	30.6	1	433	11.5
5	7	21.0	2	295	23.7
6	5	32.0	1	377	13.3
7	2	13.0	4	255	7.84
8	4	22.3	1	376	10.6
9	3	18.8	2	330	9.10
Mean ± SD[a]	4±2	22.9±10.6	2.8±1.9	363±104	11.5±6.6
Mean ± SD	4±2	21.7±8.7	2.3±1.7	346±93	11.3±5.3
3 months					
2	2.5	20.3	4	335	7.46
3	2	32.4	2	626	3.20
4	3	21.4	3	321	9.35
5	7	22.6	1	312	22.4
6	4	23.8	2	345	11.6
7	1	8.0	2	149	6.70
10	2	12.2	4	202	9.92
11	4	25.5	2	417	9.60
Mean ± SD[a]	3±2	21.4±7.9	2.3±1.0	348±154	10.1±6.6
Mean ± SD	3±2	20.8±7.6	3±1	338±144	10.0±5.6

AUC_{tau}, area under the curve for the given dose interval; C_{max}, maximum concentration; CL/F, total whole blood oral clearance; t_{max}, time to maximum concentration

[a]Patients studied on both occasions

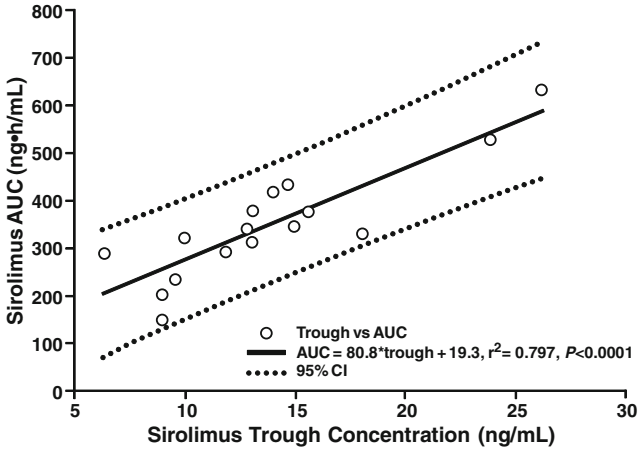

Fig. 3 Linear regression of sirolimus trough concentration versus AUC_{tau}

failed to show $C_{min,TN}$ as being a significant factor for death (OR=0.893; 95% CI, 0.710–1.124).

Discussion

Therapeutic drug monitoring of immunosuppressant medications has become an important facet of solid organ transplantation, primarily to optimize the balance between immunosuppression and a lack of adverse events associated with treatment. Because of interpatient variability, as well as pharmacokinetic variability and critical-dose characteristics of immunosuppressive agents, such as cyclosporine, tacrolimus, mycophenolate mofetil, and sirolimus, determination of the proper therapeutic windows for these medications is paramount. Therapeutic drug monitoring to predict the efficacy and the toxicity of immunosuppressant therapeutic options has been studied for decades; yet, there remains a pressing need for additional information, particularly among specific patient populations and/or immunosuppressant options.

The sirolimus concentration data were converted from immunoassay results and reported as HPLC equivalents, and the potential implications of the conversion should be noted [19]. Immunoassays cross-react with sirolimus metabolites, and different assays cross-react to different amounts. A positive bias of 20% has been observed in patients with renal allografts [20], although more recently, a negative bias was observed, with a similar magnitude for patients with either renal or hepatic allografts [19]. Pharmacokinetic studies of sirolimus in otherwise healthy subjects with hepatic impairment have shown that sirolimus clearance is decreased by 30% to 60%, depending on the degree of hepatic impairment [21]. A non-specific assay would not be able to distinguish modestly different ratios of the parent drug and metabolite that could be observed in or between individuals.

The pharmacodynamic properties of sirolimus in renal transplantation patients are well described [9–12, 22]; however, significant clinical factors may influence these parameters in the context of liver transplantation. Patients with hepatic transplants require the use of many medications, and the pharmacokinetic properties of some agents are altered in comparison to use in patients with normal hepatic function. Malireddy and colleagues demonstrated in humans, using orally administered midazolam as a CYP3A4 probe, that first-pass effects are altered in patients with hepatic transplants. Using intravenous infusion, the investigators found that hepatic clearance was unaltered, which suggested that the absorption through the gut decreased by ≈50%, corresponding to a 4.8-fold increase in biopsy CYP3A activity [15]. Zimmerman et al. showed that sirolimus clearance was decreased in adults with hepatic impairment. While patients with mild (Child–Pugh grade A) and moderate (Child–Pugh grade B) hepatic impairment showed a 31.8% and 36.1% decrease in clearance respectively, those with severe (Child–Pugh grade C) impairment showed a reduction in clearance of more than 67%. Consequently, dose reductions of 25% to 50%, as well as therapeutic drug monitoring to assess any further dose adjustments, are recommended for patients with impaired hepatic function [21, 23].

In the present study, sirolimus pharmacokinetic parameters are reported for the first time in patients who have undergone orthotopic hepatic transplantation, with a focus on the relationship between sirolimus concentrations and clinical parameters such as rejection, death, or the incidence of specific adverse events. Based on the 1-year results presented herein, variability in sirolimus trough concentrations was observed, although mean trough concentrations over time were consistent. Overall, these results are consistent with what has been observed in studies conducted in

Table 3 Mean time-normalized concentrations in liver allograft recipients experiencing events

Group	Stomatitis, ng/mL (*n*)	Pneumonia, ng/mL (*n*)	Rejection, ng/mL (*n*)	Death, ng/mL (*n*)
Event	10.2±4.91 (177)	12.0±5.48 (13)*	9.91±3.75 (41)	9.07±5.40 (9)
No event	10.2±2.90 (212)	10.1±3.03 (376)	10.2±2.80 (348)	10.0±2.92 (380)
Median time to first occurrence, days	16	170	43	140

*Significantly different from sirolimus no event (P=0.028)

patients with renal allografts [22, 24] and in healthy volunteers [25].

As noted in the results, 6 patients were studied on both occasions. In 5 of the 6, sirolimus clearance decreased by 5% to 43%, with the patient having the highest clearance (subject 5) showing the smallest decrease, and the patient with the lowest clearance on both occasions (subject 3) having the largest decrease. The reason for the difference between the day-28 and month-3 observations in the patients who were studied is not clear. The patients were well past the immediate postoperative period when changes in clearance, usually increasing with time, might occur. The differences may simply be due to random chance, the small sample size, or differences in conditions of sirolimus administration or administration of concomitant medications.

Time-normalized sirolimus trough concentrations were found to be significantly related to the development of pneumonia, although the incidence of pneumonia (n=12; 3% of patients) was relatively small. Correlation of trough concentrations with rejection, death, or stomatitis was not observed in the study population.

It should be noted that this pharmacokinetics study is limited by its small population size. Additionally, it might be expected that the patients who developed pneumonia may have been over-immunosuppressed, at least partly because of the higher levels of sirolimus, compared with patients without pneumonia. However, although the $C_{min,TN}$ for sirolimus was significantly greater in those who experienced pneumonia vs those who did not, neither value (12.0±5.48 vs 10.1±2.80) was greater than the targeted therapeutic range of 6 to 16 ng/mL for adequate immunosuppression.

It must be acknowledged that the pharmacodynamic analysis performed in this study is a simplification of what is certainly a most complex phenomenon. Only sirolimus $C_{min,TN}$ was considered. Concomitantly administered medications could potentially contribute to a combined effect of immunosuppression. Immunological match, condition of the transplanted liver, and the presence of viral infections could all potentially contribute to our understanding of the relationship between $C_{min,TN}$ and rejection. The factors that could be potentially important in understanding the contribution of $C_{min,TN}$ and death are even more complex.

In conclusion, this study demonstrated that in patients with hepatic allografts, the pharmacokinetic profile of sirolimus appears to be consistent with that previously reported for renal transplantation recipients. With the exception of pneumonia, there was no correlation between time-normalized trough concentrations of sirolimus in patients who developed significant complications, including rejection, stomatitis, and death. The maintenance of appropriate sirolimus trough concentrations remains a vital component of sirolimus therapy, particularly in terms of balancing efficacy in preventing allograft rejection against potential complications of over-immunosuppression, such as pneumonia.

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