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PHARMACOKINETICS OF THE IMMUNOSUPPRESSANT EVEROLIMUS IN MAINTENANCE RENAL TRANSPLANT PATIENTS

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Abstract: The novel macrocyclic immunosuppressant everolimus has been approved for use in renal and heart transplantation. The objective of this randomized, double-blind, placebo-controlled, dose-escalating Phase 1 study was to evaluate the pharmacokinetic profile of different dosing regimens of everolimus. Fifty-four subjects were randomized for 4-weeks treatment with everolimus (n = 44) or placebo (n = 10). Steady state was reached by day 4 of multiple dosing with evidence for dose-proportionality over the dose range tested. Systemic accumulation was 1.6- to 2.2-fold with multiple dosing. Steady-state predose trough concentrations were well correlated with AUC ($r = 0.87$, $p < 0.001$). Within-subject coefficients of variation for the tablet formulation ranged from 10-19% and between-subject coefficients from 34-60% for C_{max} and AUC. There was no effect of common demographic parameters (age, sex, weight) on variability in steady-state exposure. These results support the clinical use of everolimus in renal transplantation.

Key words: everolimus, pharmacokinetics, renal transplantation, nephropharmacology

INTRODUCTION

Despite a markedly improved graft survival over the last decades, transplant recipients remain at significant risk of acute or chronic graft rejection. The novel macrocyclic immunosuppressant everolimus (Certican™, RAD) [40-O-(hydroxy)ethyl-rapamycin] is a promising candidate for adjunctive immunosuppression [1, 2]. Everolimus has potent immunosuppressive activity and has a mode of action different to that of cyclosporine and other classes of immunosuppressants [1, 2]. Similar to sirolimus, everolimus targets the pathway involved in cell-cycle progression, and leads through general inhibition of growth-factor-dependent proliferation to the inhibition of cell proliferation, including vascular smooth muscle cell proliferation [2]. Because chronic rejection has been attributed to proliferation of vascular smooth muscle cells, a direct effect of everolimus on smooth muscle cell proliferation is expected to have an impact on long-term outcomes [1], as evidenced by a significant reduction of intimal hyperplasia in heart transplants [3].

This is the first study to investigate the tablet formulation of everolimus across a wide range of doses, unlike the Phase 1 single-dose study [4]. A previously reported multiple-dose study used in development a service capsule formulation [5] which will not be commercially available. The primary objective of this clinical study with everolimus was to assess safety and tolerability [6]. In addition, this study was designed to gather further pharmacokinetic information to support the clinical use and dosing of everolimus in renal transplantation. The aim of the present report was to explore different pharmacokinetic aspects in small cohorts of patients receiving the market tablet of everolimus. Areas explored included single- and multiple-dose proportionality, pharmacokinetic variability, drug accumulation and influence of common demographic covariates.

MATERIALS AND METHODS

This was a randomized, double-blind, placebo-controlled study in stable kidney allograft recipients. Details of the study protocol were published recently [6]. In brief, stable recipients of a primary renal transplant (cadaveric or living donor) were eligible for inclusion in the study. For at least 3 months before enrolment into the study all patients were receiving the Neoral® formulation of CsA sufficient to produce morning trough levels of 80-200 ng/mL and prednisone doses (or equivalent) at ≤15 mg/day. Subjects were aged 18-68 years, were at least 6 months post-transplant and had stable serum creatinine. It was intended to enroll sequential cohorts of 8 patients each (2 on placebo, 6 on everolimus) into escalating dose regimens; for some of the higher dose cohorts, enrollment was stopped due to poor tolerability or safety concerns. In total, 44 patients were evaluable for everolimus pharmacokinetics. The following dose regimens were assessed: 0.75, 2.5, 5, and 10 mg tablet given orally once-daily (QD); 2.5 and 5 mg tablet given orally twice-daily (BID); 0.75 mg capsule given orally QD. These regimens were administered for 28 days unless interrupted

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due to tolerability or safety concerns. *Pneumocystis carinii* prophylaxis with cotrimoxazole (480 mg/day) was mandated. All subjects gave written informed consent prior to inclusion. The study was conducted in accordance with Good Clinical Practice guidelines, and in line with the Declaration of Helsinki and European Economic Community (EEC) directives. The protocol was approved by the Ethics Committee at each center. This study was of an exploratory design; i.e. the study was not powered to address a specific statistical hypothesis.

Morning trough whole blood levels (C_{min}) of everolimus were measured at 1-3 day intervals throughout the study until day 28, a final sample was obtained at study completion on day 42. Full pharmacokinetic profiles of everolimus over a 12- or 24-hour dosing interval were obtained on days 1, 15 and 21. On profiling occasions everolimus doses were administered after an overnight fast with 250 ml water at least 60 min prior to the next meal, and blood samples were obtained from a forearm vein via an indwelling cannula predose and then 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 16, and 24h thereafter. Blood samples (1.5 ml) were drawn into EDTA-coated collection tubes, gently inverted several times, and frozen at -20 °C or below, and analyzed in duplicate in a central laboratory by a validated ELISA method [4, 5, 6]. The limit of quantification was 2 ng/ml.

Standard non-compartmental pharmacokinetic parameters were derived including the peak concentration (C_{max}) and the time of its occurrence (t_{max}), the area under the concentration-time curve over the dosing interval (calculated by trapezoidal summation; AUC_t), where is t = 24h for once-daily and 12h for twice-daily regimens. Additionally the drug accumulation ratio: $R = AUC_t^{steady\ state} / AUC(0\text{-}t)$ and the percent peak-trough fluctuation ($PTF = [(C_{max} - C_{min}) / C_{avg}] \bullet 100$) was calculated.

If samples for pharmacokinetic analysis were obtained, these were included in the results, even if the patients discontinued the study. Attainment of steady state for everolimus was assessed by linear regression analysis of the serial trough concentrations over time. A slope not significantly different from zero (a horizontal line) was taken as evidence for steady state conditions. To quantify everolimus inter- and intra-patient pharmacokinetic variability, the replicate steady state parameters from days 15 and 21 were dose-normalized and assessed in a two-way ANOVA with patient and replicate as sources of variation. This was performed separately for each dose level. The absence of a significant *Day*-effect was taken as evidence of steady-state on the profiling occasions. The respective coefficients of variation were derived from the standard deviation (square root of the respective mean square term) divided by the global mean of the parameter. The replicate determinations of C_{max}, C_{min}, AUC_t, and PTF from Days 15 and 21 were compared in a repeated measures ANOVA with *Subject* and *Replicate* as sources of variation. The mean square from the Error term was taken as a measure of the intrasubject variance and the mean square from the Subject term as a measure of intersubject variance.

The influence of weight and age on C_{max} and AUC_t was explored by linear regression. The fraction of variability in the parameter explained by the demographic covariate was based on the coefficient of determination (r^2-value). The influence of sex was explored by unpaired t-test.

Results

44 subjects were randomized for treatment with everolimus 10 patients received placebo. Because the highest dosage group was discontinued due to safety concerns [6], only seven dose regimens were assessed: 0.75 mg (everolimus n = 6; placebo n = 2), 2.5 mg (everolimus n = 6), 5 mg (everolimus n = 6; placebo n = 2), and 10 mg (everolimus n = 2; placebo n = 1) tablet given orally once-daily (QD); 2.5 mg (everolimus n = 12; placebo n = 2), and 5 mg (everolimus n = 6) tablet given orally twice-daily (BID); 0.75 mg (everolimus n = 6; placebo n = 2),) capsule given orally QD. The main demographic characteristics of the study patients and background medical characteristics were comparable across treatment groups [6]. It is important to note, that the population was predominantly Caucasian (50/54), with only 4 Asian/Oriental patients. A total of 44 everolimus pharmacokinetic profiles were evaluable for day 1 (first dose), and 32 and 30 steady state profiles were evaluable for days 15 and 21, respectively.

Drug Accumulation and Attainment of Steady State

For patients receiving the tablet formulation of everolimus twice daily, single-dose and steady state (multiple-dose) pharmacokinetic parameters for each dose group are summarized in Table 1. Comparing the AUC from Days 15 and 21 with that from Day 1 yielded mean accumulation ratio of 1.8 from the 2.5 mg BID regimen with individual ratios ranging from 1.2 to 2.8. Because the 5 mg twice-daily regimen was discontinued, there were insufficient steady state data to include this dose level in the evaluation. Accumulation ratios for the QD regimens were similar: Individual ratios ranged from 1.2 to 2.6 with mean values between 1.6 to 2.2 across the 0.75 mg to 5 mg QD regimens. Accumulation ratios appeared independent of dose level. In general, there is a 1.6- to 2.2-fold systemic accumulation of everolimus to steady state.

Regression analysis was not performed on the troughs from patients receiving the capsule formulation since many of the data points were below the assay limit of quantification due to low systemic exposure from this formulation. A synoptic view of individual trough trajectories over the study duration from patients receiving the tablet formulation are shown in Figure 1. In general, steady state was reached by day 4 indicating an accumulation half-life of approximately 24 hours. Lack of steady state was apparent for 3 patients in the higher dose levels (2.5 mg bid and 5 mg bid) whose troughs declined a few days before withdrawing from the study due to poor tolerability. It is likely that they were not compliant just prior to their study discontinuations. Their last

Table 1. Everolimus pharmacokinetics in twice-daily regimens.

Parameter	2.5 mg bid Day 1 (n = 12)	Day 15 (n = 9)	Day 21 (n = 7)	5 mg bid Day 1 (n = 6)	Day 15 (n = 1)
t_{max} (h)	1.3 (0.5-2.5)	1.0 (0.8-2.5)	1.0 (0.8-2.5)	1.3 (0.8-1.5)	2.5
C_{max} (ng/ml)	57.1 ± 23.6	70.4 ± 19.7	68.6 ± 22.3	110 ± 28	109
C_{max}/Dose (ng/ml/mg)	22.8 ± 9.4	28.2 ± 7.9	27.4 ± 8.9	22.0 ± 5.7	21.8
AUC_t (ng*h/ml)	234 ± 79	398 ± 103	378 ± 123	462 ± 73	803
AUC_t/Dose (ng*h/ml/mg)	94 ± 31	159 ± 41	151 ± 49	92 ± 15	161
C_{min}(ng/ml)	--	24.3 ± 11.3	25.3 ± 16.2	--	47.6
C_{avg} (ng/ml)	--	33.2 ± 8.6	31.5 ± 10.2	--	67.0
PTF (%)	--	141 ± 35	144 ± 42	--	92

Values are mean ± SD; t_{max} is median (range). Individual values are given for 5 mg bid

Table 2. Everolimus pharmacokinetics: once-daily regimens at steady state.

Parameter	0.75 mg qd		2.5 mg qd		5 mg qd	
	Day 15 (n = 4)	Day 21 (n = 5)	Day 15 (n = 3)	Day 21 (n = 3)	Day 15 (n = 6)	Day 21 (n = 5)
t_{max} (h)	1.5(1.5-2.0)	1.5(1.0-2.5)	1.0(1.0)	1.0(1.0-1.5)	1.0(1.0-1.5)	0.8(0.8-1.5)
C_{max} (ng/ml)	21.1 ± 3.7	21.6 ± 4.2	46.8 ± 14.9	62.1 ± 13.1	118 ± 32	114 ± 36
C_{max},/Dose (ng/ml/mg)	28.1 ± 4.9	28.8 ± 5.6	18.7 ± 6.0	24.8 ± 5.3	23.5 ± 6.4	22.8 ± 7.2
AUC_t (ng*h/ml)	213 ± 62	228 ± 87	324 ± 99	570 ± 195	886 ± 311	716 ± 123
AUC_t/Dose (ng*h/ml/mg)	284 ± 82	304 ± 116	129 ± 40	228 ± 78	177 ± 62	143 ± 25
C_{min}, (ng/ml)	4.8 ± 1.0	5.1 ± 1.7	6.8 ± 2.5	13.6 ± 9.0	18.3 ± 9.0	17.4 ± 4.5
C_{avg}, (ng/ml)	8.9 ± 2.6	9.5 ± 3.6	13.5 ± 4.1	23.7 ± 8.1	36.9 ± 12.9	29.8 ± 5.1
PTF (%)	188 ± 28	194 ± 81	303 ± 136	215 ± 50	282 ± 70	321 ± 78

Values are mean ± SD; t_{max} is median (range). For 2.5 mg qd, n=3 because the other 3 patients on each day were nonfasting.

pharmacokinetic profile was omitted from evaluation as being non-steady state. A further confirmation that the Day 15 and 21 profiles were at steady state was demonstrated by the ANOVA on the concentrations at the beginning and end of the two profiles. There were no significant differences in any of the dosing cohorts.

Dose-proportionality

Exposure from BID regimens was consistent with dose-proportionality inasmuch as dose-normalized C_{max} and AUC on Day 1 did not differ between dose levels (p = 0.92 and 0.84, respectively). Insufficient data for the higher dose regimen precluded testing for dose-proportionality at steady state.

Dose-proportionality in everolimus C_{max} and AUC_{24} over the single dose range 0.75 to 10 mg from QD regimens was indicated by lack of differences among dose-normalized parameters (p = 0.46 and 0.27, respectively) and significant regressions with y-axis intercepts not significantly different from zero. The regressions yielded the following equations: C_{max} = 16.0 • Dose + 8.9 (p = 0.0001 for slope; p = 0.25 for intercept); AUC_{24} = 97 • Dose + 46 (p = 0.0001 for slope; p = 0.48 for intercept).

Because the 10 mg once-daily regimen was discontinued, there were insufficient steady state data to include this dose level in the evaluation. Therefore, steady state dose-proportionality was assessed from 0.75 to 5 mg once daily (Table 2). Both ANOVA and linear regression demonstrated a dose-proportional rise in C_{max} for days 15 and 21 as well as for combined data from both days. The slopes (p = 0.72) and intercepts (p = 0.81) from regression analysis on the two profiling days were not significantly different; hence, all steady-state data were combined for a pooled regression yielding the following equation: C_{max} = 22.4 • Dose + 2.8 (p = 0.0001 for slope; p = 0.73 for intercept).

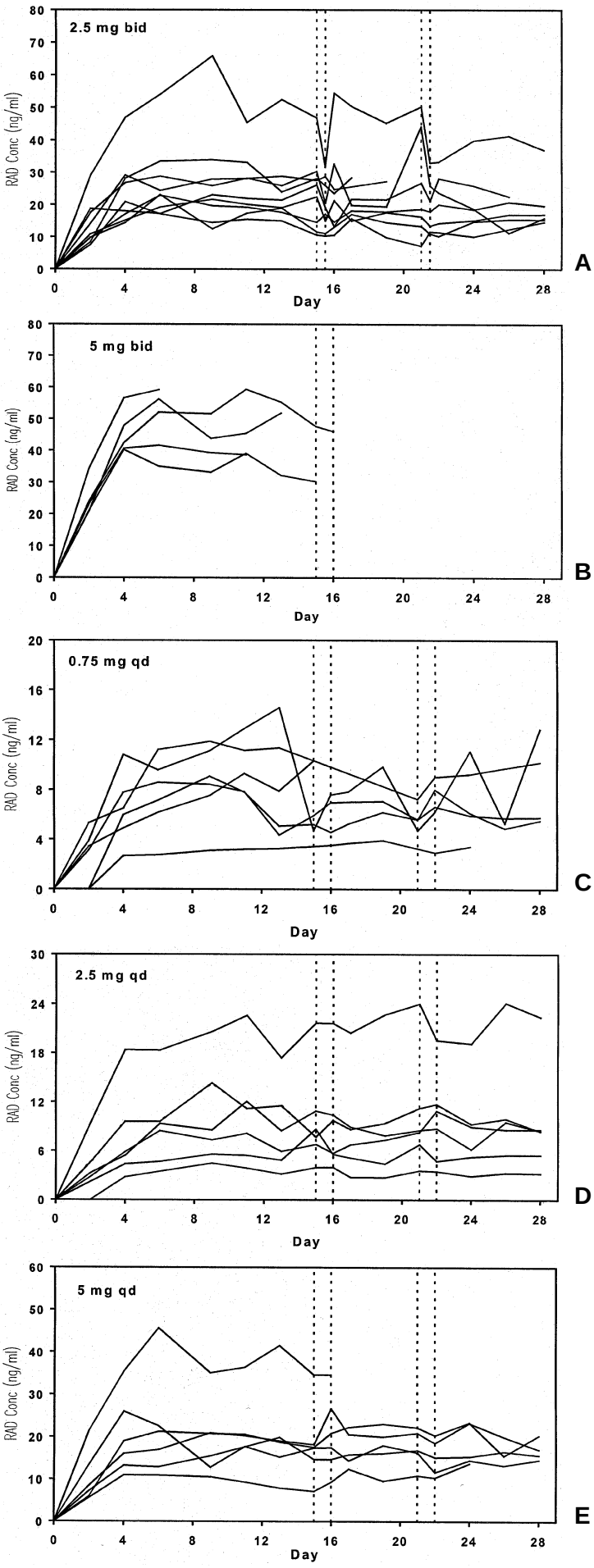

Fig. 1. Serial everolimus (RAD) predose trough concentrations for subjects receiving everolimus A) 2.5 mg BID; B) 5 mg BID; C) 0.75 mg QD; D) 2.5 mg QD; E) 5 mg QD. Dashed lines indicate profiling occasions on Days 15 and 21. Included are data up to the day of everolimus discontinuation.

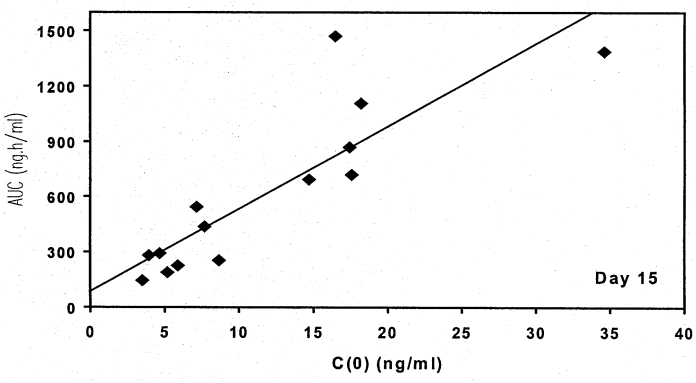

Fig. 2. Correlation between everolimus (RAD) trough concentration and AUC at steady-state on day 15: AUC = 44.8 x C(0) + 84.2 p = 0.0001 (slope) r = 0.864

On both steady-state profiling occasions, dose-normalized AUC_{24} differed among the dose levels (p = 0.02 and 0.03 on Days 15 and 21, respectively). Pairwise contrasts indicated that the differences existed between 0.75 mg and the higher dose levels; whereas, the 2.5 and 5 mg levels did not differ. Both ANOVA and linear regression on AUCs indicated that values in steady state from the 2.5 and 5 mg once-daily regimens rose in a dose-proportional manner but that the data at 0.75 mg was slightly higher than expected for dose-proportionality in this parallel-group assessment. All steady-state data were combined for a pooled regression yielding the following equation which was in support of dose-proportionality for this parameter at steady state: AUC_{24} = 139 • Dose + 112 (p = 0.0001 for slope; p = 0.12 for intercept).

Steady-state predose trough concentrations [C_{min}] were significantly correlated with AUC_{24} from once-daily regimens of 0.75 mg to 10 mg on Day 15 (r = 0.86) as illustrated in Figure 2, as well as on Day 21 (r = 0.95).

Intra- and Intersubject Pharmacokinetic Variability and Covariates

Coefficients of pharmacokinetic variability at steady state were 10-19% within-patient and 34-60% between-patient (Table 3). Within-subject coefficients of variation ranged from 9.6% to 18.6% and did not appear to differ with formulation (tablet or capsule), regimen (once- or twice-daily), or the pharmacokinetic parameter. Between-subject coefficients of variation (with the exception of C_{min}) ranged from 30.7% to 59.8% regardless of formulation and regimen. Only C_{min} exhibited somewhat higher variations in excess of 60%.

Variability in steady-state exposure (AUC_t standardized to 24 h and dose-normalized) from common demographic covariates was assessed in 23 patients receiving the tablet formulation on Day 15. Neither weight (AUC/Dose = 0.16 x Weight + 171 p=0.88 (slope) r^2 = 0.001) nor age (AUC/Dose = 0.88 x Age + 139 p=0.55 (slope) r^2 = 0.01) contributed significantly to the variability in everolimus exposure. Likewise, exposure was not different between sexes: 165 ± 50 (women, n = 10) versus 193 ± 84 (men, n = 13) ng • h/ml per mg (p = 0.43; not shown).

Table 3. Everolimus intra- and intersubject variability of tablet formulation in QD and BID regimens.

Parameter	Tablet (QD)		Tablet (BID)	
	%CVw	%CVb	%CVw	%CVb
C_{max}	9.6	33.8	18.6	39.0
AUC_t	14.4	59.8	15.8	38.1
C_{min}	11.2	61.0	18.6	83.2
PTF	17.6	43.6	17.8	36.3

%CV: percent coefficient of variation within (w) and between (b) subjects.

Discussion

Several clinical pharmacokinetic aspects of everolimus were explored in the present investigation as a general guide for its use in ongoing clinical studies. Everolimus daily dosages over a large dose range from 0.75 mg daily up to 10 mg daily were assessed. Only the tablet formulation of everolimus, which will be the marketed form, was used in the higher dosage groups. This is of particular importance, as the everolimus market tablet had a 2.6 times higher bioavailability compared with the service capsule [6]. Everolimus was administered as add-on therapy to maintenance renal transplant patients on a double immunosuppressive regimen consisting of CsA microemulsion and steroids. The characteristics of subjects enrolled in the study match those of the intended target population. Here we report the detailed pharmacokinetic results of the first, multiple oral dose clinical study of everolimus tablets. In addition to the conventional characterization of single- and multiple-dose profiles, data were explored to characterize everolimus accumulation kinetics, to gather evidence of dose-proportionality, pharmacokinetic variability, the influence of common demographic covariates, and to assess the ability of the predose trough concentration to reflect the full exposure over the dosing interval at steady state.

Several aspects of everolimus pharmacokinetics were explored in the present study to provide a platform for ongoing and future trials. Clinically robust oral formulations of rapamycin are difficult to produce [7]. By contrast, oral absorption of everolimus from

the market tablet formulation was rapid and consistent. This was the first study of different everolimus formulations, and it demonstrated that the tablet formulation promoted efficient and reliable absorption of everolimus. This latter aspect was demonstrated by low intrasubject, interoccasion variability for everolimus steady-state pharmacokinetic parameters with coefficients of variation between 10% and 19%. The time taken to reach steady state levels of everolimus and drug accumulation at these levels were consistent with the half-life of everolimus (approximately 24-30 hours) in earlier studies which for everolimus is shorter than that reported for sirolimus [4, 8].

Another important objective of the present study was to evaluate dose-proportionality across the dose range studied. Single-dose profiles on Study Day 1 yielded evidence for dose-proportionality over the dose range 0.75 to 10 mg tablets. These results are consistent with those of a previous study [5] in which dose-proportionality was demonstrated over the range 0.75 to 7.5 mg of the service capsule. Also in agreement with this study [5], the drug accumulation with multiple dosing was around 2-fold or less and steady state was attained by Day 4 of once- and twice-daily dosing. This is also consistent with everolimus half life in adult renal transplant recipients of 25-35 h [4]. The picture for dose-proportionality at steady state (Days 15 and 21) was less clear than that after single dosing. Specifically, ANOVA detected higher dose-normalized C_{max} and AUC_{24} from 0.75 mg compared with 2.5 and 5 mg given once-daily. It is noteworthy that these metrics were similar at 2.5 and 5 mg. Since patients did not cross over among the different dose levels, the higher exposure at the lowest dose level may have been due to between-group variability in this parallel-group assessment. Because the slopes and intercepts from regressions on Days 15 and 21 did not differ significantly, data were combined for a composite regression which was consistent with steady-state dose-proportionality over the range 0.75 to 5 mg once daily. It is noteworthy that dose-proportionality was found at steady state for the service capsule in a similar patient population [5].

This dose escalating phase I study provided evidence, that everolimus up to 5 mg daily has an acceptable safety profile, while doses of 10 mg are beyond tolerability [6], thus extending our knowledge on this new immunosuppressive compound. Everolimus doses up to 5 mg daily were found to be adequately well tolerated. Adverse events frequently reported in everolimus-treated patients [2,6] included hypercholesterolemia (16%), thrombocytopenia (34%), anemia (14%), aphthous stomatitis (14%), and extrasystoles (14%) i.e. the AE profile observed largely reflected the profile established for the class of macrolide immunosuppressants to which everolimus belongs [2, 8]. As a consequence everolimus daily doses of up to 4 mg were used in the phase II and III studies [9, 10].

Conclusion

This study provides the first evidence that multiple daily dosing of everolimus, in doses up to 5 mg/day is adequately well tolerated as add-on therapy in stable renal transplant patients receiving maintenance cyclosporine immunosuppression. The steady state pharmacokinetic profiles of everolimus showed dose-proportionality across the dose range studied, and variability was low. Everolimus concentrations accumulated 1.6- to 2.2-fold with multiple dosing and reached steady state by day 4 of once- and twice-daily administration.

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