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ORIGINAL ARTICLE

A randomized cross-over comparison of short-term exposure of once-daily extended release tacrolimus and twice-daily tacrolimus on renal function in healthy volunteers

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Summary

Calcineurin inhibitor nephrotoxicity remains an issue for transplant recipients. The pharmacokinetic profile (PK) of the once-daily tacrolimus extended release (Tac-ER) includes equivalent exposure [$AUC_{(0–24\ h)}$] but lower C_{max} versus twice-daily tacrolimus immediate release (Tac-IR). We hypothesized that the unique PK profiles would result in pharmacodynamic differences in renal function. Nineteen healthy male subjects were allocated to once-daily Tac-ER and twice-daily Tac-IR in a prospective, randomized, two period, cross-over study. Tacrolimus was titrated to achieve trough levels of 8–12 ng/ml. Twenty four hours ERPF and GFR estimated by para-aminohippurate and sinistrin clearance were performed at baseline and at the end of each 10-day dosing period. Mean Tac C_0 was 11.0 ± 2.2 and 11.3 ± 1.8 ng/ml for Tac-ER and Tac-IR, respectively. The mean Effective 24 h renal plasma flow (ERPF) was significantly higher with Tac-ER compared with Tac-IR (658 ± 127 vs. 610 ± 93 ml/min/1.73 m^2, $P = 0.046$). There was a trend to a greater mean GFR over 24 h for Tac-ER at 114.5 ± 13.6 ml/min/1.73 m^2 compared with 108.9 ± 9.7 ml/min/1.73 m^2 for Tac-IR, $P = 0.116$. Under controlled physiological conditions, ERPF was significantly improved with Tac-ER compared with Tac-IR, likely owing to the differing PKs of these tacrolimus preparations (ClinicalTrials.gov Identifier: NCT01681134).

Introduction

Calcineurin inhibitors (CNI) have remained the cornerstone of immunosuppression regimens for recipients of solid organ transplants for more than 30 years. Despite the excellent graft and patient survival seen in the modern immunosuppressive era, CNI nephrotoxicity remains the major limitation of these agents [1,2]. The hemodynamic effects of CNI exposure are well described, with peak plasma cyclosporine microemulsion (CsA) levels correlating with decreases in both effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) [1,3–5]. Furthermore, therapeutic doses of CsA induce a greater reduction in renal perfusion compared with tacrolimus immediate release (Tac-IR), suggesting Tac-IR confers a lower risk of renal ischemia [6].

A once-daily formulation of tacrolimus (Tac-ER) has been developed and widely adopted by renal transplant

centers across the world based on efficacy and the potential for improved adherence [7–10]. The steady state 24-h exposure [$AUC_{(0–24\ h)}$] and trough level (C_0) of once-daily Tac-ER are equivalent to conventional twice-daily Tac-IR but with a differing pharmacokinetic (PK) profile with the potential to further improve the renal hemodynamic profile of CNIs [10]. In particular, there is a reduced C_{max} compared with Tac-IR and only a single peak. We have recently demonstrated in healthy volunteers under controlled conditions that the reduced peak exposure observed with Tac-ER resulted in both improved renal blood flow and GFR compared with CsA [11].

To determine whether PK profile differences between Tac-ER and Tac-IR produce different physiological effects, we set out to compare the renal hemodynamic function profiles associated with these agents in healthy study participants with normal renal function in a controlled laboratory setting using gold-standard measures of renal blood flow and GFR. We hypothesized that single daily dosing with Tac-ER (Advagraf®, Astellas Pharma, Canada Inc.) would improve renal perfusion compared with the twice-daily Tac-IR (Prograf®, Astellas Pharma, Canada Inc.).

Methods

Design and participants

This was a prospective, single center, randomized, open-label, concealed allocation, 2-period, 2-sequence, single cross-over study, with a primary objective to describe the renal pharmacodynamic (PD), and tacrolimus PK profiles during 24-h steady-state dosing intervals and determine the presence of temporal associations between PK and PD for Tac-ER and Tac-IR in healthy male adult volunteers. Pharmacodynamic is defined as ERPF and GFR. The research study was conducted at INC Research Toronto, Ontario, Canada, under International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Consolidated Guideline and all applicable regulatory requirements and was approved by the local IRB. To be eligible for the study, subjects had to be provided with and sign a written consent form, and be nonsmoking, nonhypertensive Caucasian males aged between 18 and 45 years with a body mass index between 19 and 27 kg/m^2. Subjects had to be healthy (including normal laboratory results) with no history of renal dysfunction, serious head injury, epilepsy or eating disorders; have a normal ECG, a negative drug screen; and be willing to abstain from alcohol for the study duration.

Eligible subjects were randomized using a schedule generated by INC's validated proprietary computer software program to one of two study sequences (Tac-ER–Tac-IR or Tac-IR–Tac-ER). Each dosing period (Tac-ER or Tac-IR) lasted for 10 days and were not separated by a washout period (i.e. total Tac exposure was 20 days). At baseline and the final day of each dosing period, subjects were confined to the INC clinical research facility for 24-h renal function studies. Subjects engaged in standardized physical activity and strenuous activity was prohibited at all times during the confinement.

CNI dosing

Tacrolimus was administered at an initial morning dose of 0.15 mg/kg in one undivided dose for Tac-ER or 0.075 mg/kg bid (administered in the morning and 12 h later) for Tac-IR. Therapeutic drug monitoring (TDM) was performed every second day and doses were adjusted to achieve a target C_0 of 8–12 ng/ml, similar to early exposure TDM in kidney transplant recipients.

Study preparation

Participants were maintained on a sodium-replete (target daily intake of >140 mmol/day) and moderate protein (daily intake of 1–1.5 g/day) diet 7 days prior to baseline and until end-of-study visit. The sodium-replete diet was used to avoid effective circulating volume contraction and activation of the renin angiotensin aldosterone system [12]. Study drug administration was standardized with respect to meal content and consumption, at least 2 h before or after a meal. All breakfasts consumed during the confinements were standardized to 850 Kcal (30% fat, 16% protein, and 54% carbohydrate). All other meals consumed during the confinements were standardized to approximate the aforementioned proportions of fat, protein, and carbohydrate content across all drug dosage interval days and interval sequences.

Physiological assessments

All tests of renal function were done at baseline (prior to drug exposure) and on the 10th day of exposure to Tac-ER or Tac-IR. GFR and ERPF were determined by the intravenous (IV) administration of sinistrin, an analog of inulin and para-aminohippurate (PAH), respectively, as described below and in our previous publications [12–14]. The ERPF and GFR assessments were performed over a 24 h period, using plasma concentrations of inulin and PAH, at the times described below. During the renal physiology assessments, subjects were required to remain supine except to void.

Renal hemodynamic function tests were performed starting at approximately 0800 hours. A first intravenous line was inserted into the left arm for drawing blood, and a second intravenous line was inserted into the right arm and connected to a syringe infusion pump for infusions of sinistrin and PAH. Peripheral blood pressure was measured in

the right brachial artery with an automated DINAMAP® sphygmomanometer (Critikon, Tampa, FL, USA) prior to each blood sample throughout the study. After collecting blood for sinstrin and PAH blank, a priming infusion containing 25% sinistrin (60 mg/kg) and 20% PAH (8 mg/kg) was administered. Thereafter, sinistrin and PAH were infused continuously at a rate calculated to maintain their respective plasma concentrations constant at 20 and 1.5 mg/dl, respectively. After a 90-min equilibration period, blood was collected for sinistrin, PAH, and hematocrit (HCT), and thereafter at 0 h (the time of CNI administration, except for baseline), 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 10, 12, 13, 14, 15, 16, 18, 20 and 24 h. This methodology obviates the need for any urine sampling. Blood pressure measures were also taken simultaneously at these time points [12]. All experiments were performed in the same warm (25 °C), temperature-controlled room, and in a quiet environment [12].

Study assays

Pharmacokinetic

Determinations by reference HPLC-MS:MS of Tac levels for the purpose of PK analyses were conducted at St. Michael's Hospital Core Laboratory on whole blood specimens collected at 0 h (the time of CNI administration), 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 10, 12, 13, 14, 15, 16, 18, 20 and 24 h postdose on the PD/PK renal physiology assessment days. Infusion of PAH and sinistrin was stopped prematurely, 1–2 h before the 24 h samples in all subjects. This was an error in the study process, having only been uncovered following completion of all study subject evaluations. As the 24-h data points of ERPF and GFR were not usable, model-based imputations were performed for the missing 24 h samples; the 24 h ERPF and GFR data points were predicted using a mixed model with repeated measures on the last three data points (16, 18, 20 h data points) and predose (0 h) data points, replaced for 24 h values.

Renal hemodynamic function assays

Blood samples collected for sinstrinand PAH determinations were immediately centrifuged at 603 g for 10 min at 4 °C. Plasma was separated, placed on ice, and then stored at −70 °C. Inulin and PAH were measured in serum by colorimetric assays using anthrone and N-(1-naphthyl) ethylenediamine, respectively [12]. Filtration fraction (FF) was determined as the ratio of GFR to ERPF. Renal blood flow (RBF) was calculated by dividing the ERPF by (1-HCT). Renal vascular resistance (RVR) was derived by dividing the MAP by the RBF. All renal hemodynamic measurements were adjusted for body surface area (BSA) expressed per 1.73 m^2.

Neurohormonal mediators

To elucidate possible mechanisms underlying differences in the effect of Tac-IR versus Tac-ER on renal hemodynamic function, plasma levels of aldosterone, nitric oxide, and prostaglandin F1α were measured at baseline and again 2 h after drug administration. Aldosterone was measured by radioimmunoassay, using the Coat-A-Count system [12]. Nitric oxide levels were measured using a standard assay (NO, R&D Systems, Minneapolis, MN, USA -total NO/Nitrite/Nitrate assay kit, Cat No.KGE001). Prostaglandin F1α was measured as described in previous work [15].

Statistical analysis

Demographics, pretreatment characteristics and safety analyses were calculated using the intent to treat (ITT)/safety population, defined as all randomized subjects who received at least one dose of study treatment. The per protocol (PP) population, comprised of subjects who completed the study and completed all three renal and both PK assessments, was used for PK and PD analyses. Approximately 20 subjects were planned to undergo baseline renal physiology assessments to yield a minimum sample size of 15 subjects in the PP dataset. With a sample size of 15 subjects, the study would be able to detect a correlation coefficient of 0.65 or greater between PK-PD parameters at a type I error of 5% (no adjustment for multiple testing was planned), and an effect size of 0.78 between the two formulations where effect size was defined as (mean Tac-ER—mean Tac-IR)/SD; and SD is the population standard deviation.

Individual and mean values for tacrolimus concentration, and each PD variable, versus time curves were produced for Tac-ER and Tac-IR. From these descriptive statistics (mean, geometric means, standard deviation, coefficient of variation, median, minimum and maximum values) of the measured and derived PK/PD parameters (C_0, C_{max}, $T_{max,}$ and $AUC_{(0–24\ h)}$ for PK; $AUC_{(0–24\ h)}$ for PD) were calculated. Determinations of PK and PD parameters over the course of the 24 h postdose renal physiology assessment, including $AUC_{(0–24\ h)}$ were conducted by standard noncompartmental methods using WinNonlin™ version 5.2.

The treatment effect of the two formulations on the $AUC_{(0–24\ h)}$ for ERPF and GFR was tested by analysis of variance (ANOVA) using the 2 × 2 cross-over model at Day 10: Tacrolimus blood concentrations = Sequence + Subject(Sequence) + Treatment + Period. In addition, paired *t*-tests were performed to test the differences between Baseline versus Tac-ER and Baseline versus Tac-IR (regardless of treatment sequence/period). Statistical analysis was performed using SAS (Statistical Analysis Software, Cary, NC, USA) version 9.2.

Results

Baseline characteristics

A total of 19 study participants met inclusion and exclusion criteria and were enrolled, and are included in the ITT population. Two participants withdrew prior to completion and were excluded from the PP population. Of the remaining 17 subjects who completed all three renal function assessments, one participant exhibited nonphysiologic ERPF and RBF data at the Tac-IR predose and 0.5 h time points. These data points were replaced by using 1 h postdose values carried backwards.

As per enrollment criteria, only male subjects were included; their age varied between 23 and 45 years with mean (SD) of 35 (6.8), all of them were Caucasians, body mass index ranged between 19 and 29 kg/m^2 with mean (SD) of 24 (2.8) kg/m^2. Baseline demographics of the study participants are shown in Table 1.

Tacrolimus pharmacokinetics

Treatment compliance was 100% as confirmed by direct supervision. While Tac-IR C_0 levels were somewhat higher than Tac-ER at days 3 and 5, by design, steady state C_0 [tac] was achieved and were similar from Day 7 to Day 10 of the dosing periods. Mean (SD) TDM Day 10 Tac C_0 levels were 11.0 (2.2) and 11.3 (1.8) ng/ml for Tac-ER and Tac-IR, respectively ($P = 0.99$; Target: 8–12 ng/ml). Day 10 doses required to achieve these concentrations were 0.137 (0.04) and 0.119 (0.04) mg/kg/day for Tac-ER and Tac-IR, respectively (Table 2). As illustrated in Fig. 1, there was a nonsignificant higher mean (SD) $AUC_{(0–24\ h)}$ with Tac-ER (410 ± 66 h ng/ml) compared with Tac-IR (385 ± 79 h ng/ml; $P = 0.326$). Median T_{max} occurred at 1.5 h postdose, with a mean (SD) C_{max} of 32.8 (5.7) ng/ml for Tac-ER and 36.3 (7.1) ng/ml for Tac-IR. Given the twice-daily exposure for Tac-IR, a second peak with Tac-IR occurred at a median of 15 h (3 h after second dose). The complete 24 h pharmacokinetic profile is illustrated in Fig. 2.

Table 1. Subject demographics ($n = 19$). Results are mean ± SD unless otherwise stated.

Age (years)	35.4 ± 6.82
Male gender	19 (100%)
BMI (kg/m^2)	23.9 ± 2.8
Caucasians	19 (100%)
BP (mm/Hg)	122/76 ± 8.5/7.0
MAP (mm/Hg)	87.8 ± 9.9
Plasma creatinine (μmol/l)	79.2 ± 10.5

Renal hemodynamic function

The primary outcomes of 24-h ERPF, RBF and GFR were determined for the PP population at baseline and at the end of each treatment period for Tac-ER and Tac-IR utilizing 17 timed samples over the 24-h period. As illustrated in Table 2 and Fig. 3, the ERPF was significantly greater for Tac-ER compared with Tac-IR ($P = 0.046$). Although the study design did not support direct comparison between results from a treatment period and baseline, ERPF trended higher for Tac-ER compared with baseline (Mean increase: 8.7%; $P = 0.07$). The change in ERPF from baseline over the 24-h assessment period is illustrated in Fig. 4. A significant treatment effect was also observed with RBF values significantly higher for Tac-ER ($P = 0.037$ for Tac-ER versus Tac-IR; Table 2). Although there was a trend for higher treatment effect on GFR (Fig. 5 and Table 2) with Tac-ER, this did not reach statistical significance ($P = 0.116$).

Effective renal plasma flow was more fully evaluated and compared at each of the sampling times. As shown in Fig. 3, under all three study conditions, there was expected physiological nocturnal decline in both ERPF and GFR. In

Table 2. Pharmacokinetic and Pharmacodynamic Parameters. Note all values are mean ± SD. $n = 17$.

	Baseline	Tac-IR	Tac-ER
Tacrolimus dose on study day (mg/kg/dy)	–	0.119 ± 0.04	0.137 ± 0.04
Therapeutic drug monitoring day 10 tacrolimus [C_0] (ng/ml)	–	11.3 ± 1.8	11.0 ± 2.2
Tacrolimus $AUC_{(0–24\ h)}$ (hr ng/ml)	–	385 ± 79	410 ± 66#
ERPF (24 h ml/min/1.73 m^2)	14 536 ± 1724	14 643 ± 2239	15 796 ± 3039*
Average ERPF over 24 h (ml/min/1.73 m^2)	606 ± 72	610 ± 93	658 ± 127
RBF (24 h ml/min/1.73 m^2)	24 953 ± 3189	24470 ± 3169	26 564 ± 4778**
Average RBF over 24 h (ml/min/1.73 m^2)	1040 ± 133	1020 ± 132	1107 ± 199
GFR (24 h ml/min/1.73 m^2)	2626 ± 195	2614 ± 232	2747 ± 326†
Average, GFR over 24 h (ml/min/1.73 m^2)	109.4 ± 8.1	108.9 ± 9.7	114.5 ± 13.6
FF (24 h)	4.4 ± 0.4	4.4 ± 0.7	4.3 ± 0.7
Renal vascular resistance (RVR) (24 h L/min/mmHg/1.73 m^2)	2.02 ± 0.34	2.09 ± 0.42	1.95 ± 0.45

#$P = 0.326$, *$P = 0.046$, **$P = 0.037$, †$P = 0.116$, Tac-ER versus Tac-IR.

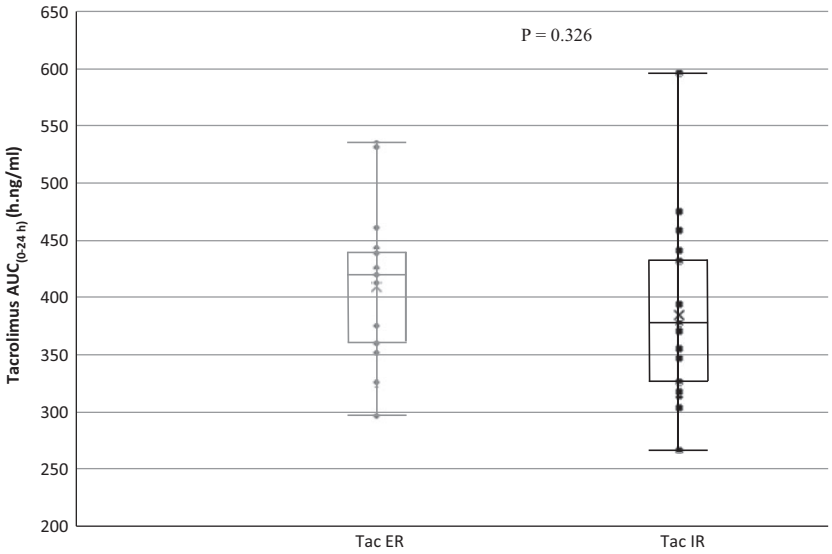

Figure 1 Box plots for tacrolimus $AUC_{(0–24\ h)}$(hr ng/ml) for Tac-ER and Tac-IR. $n = 17$. ($P = 0.326$).

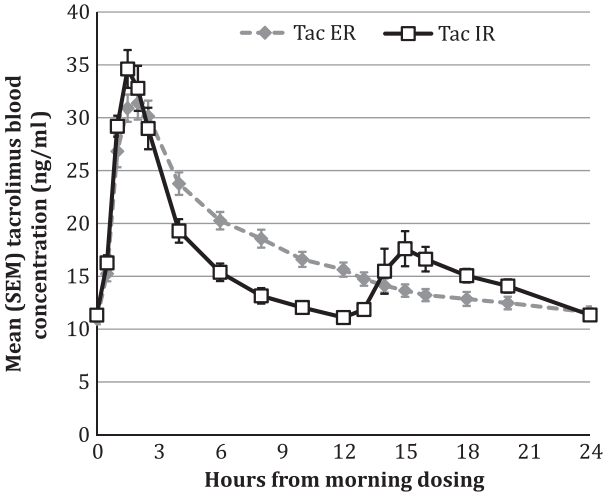

Figure 2 Mean (SEM) 24 h tacrolimus blood concentration versus time profile in ng/ml on day 10 of study, Tac-ER (dashed line) and Tac-IR (solid line), $n = 17$.

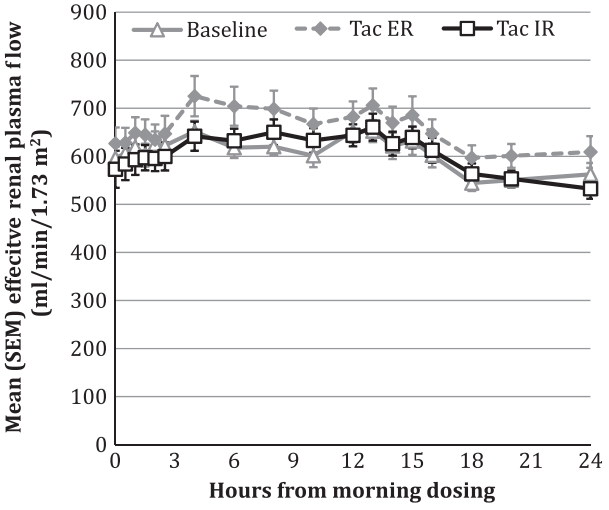

Figure 3 Mean (SEM) effective renal plasma flow (ERPF; ml/min/1.73 m^2) versus time at baseline (no treatment) (solid gray line), Tac-ER (dashed gray line), and Tac-IR (solid black line) ($P = 0.046$), $n = 17$.

addition, there was a decrease in ERPF compared with baseline, coincident with the peak exposure of Tac-IR between 1 and 4 h post-am dose. In contrast, this effect was not observed with Tac-ER. Of interest, there was an improvement in ERBF with Tac-ER compared with baseline between 3 and 10 h postdose, and a slight trend to higher ERBF with Tac-IR compared with baseline for the same time period (Fig. 4). Throughout all time periods, mean RBF was greater with Tac-ER compared with Tac-IR. There was a similar but nonsignificant trend seen with GFR (Fig. 5). As shown in Table 2, RVR and filtration fraction (FF) did not differ significantly under the three study conditions. Plasma creatinines measured at baseline (no CNI), following 10 days of Tac-IR exposure, and following 10 days of Tac-ER exposure were not different at 79.2 ± 10.5, 82.6.±9.06, 81.4 ± 6.83 μmol/l, respectively.

Plasma biochemistry and neurohormones

All biochemical parameters including: serum electrolytes, glucose, calcium, phosphate, albumin, and serum lipid profile were not different from baseline following 10-day exposure to either tacrolimus formulation. Plasma aldosterone, prostaglandin F1α, and nitric oxide levels were similar after

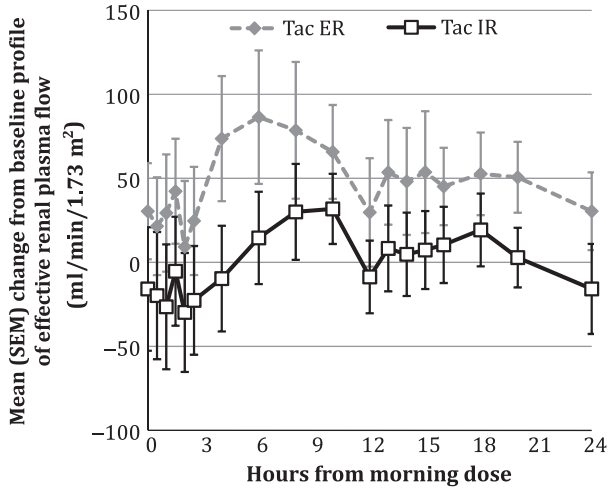

Figure 4 Mean (SEM) time-matched change from baseline (no treatment) in effective renal plasma flow (ERPF; ml/min/1.73 m^2) versus time for Tac-ER (dashed line) ($P = 0.07$) and Tac-IR (solid line) ($P = 0.677$), $n = 17$.

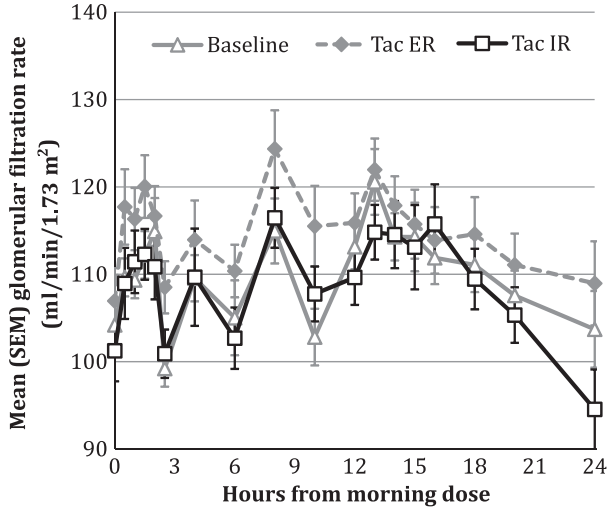

Figure 5 Mean (SEM) effective glomerular filtration rate (GFR; ml/min/1.73 m^2) versus time at baseline (no treatment) (solid gray line), Tac-ER (dashed gray line), and Tac-IR (solid black line) ($P = 0.116$), $n = 17$.

treatment with Tac-IR versus Tac-ER. Treatment with Tac-IR versus Tac-ER did not influence acute nitric oxide or aldosterone responses during inpatients study days at 2 h post-CNI. In contrast, the decline prostaglandin F1α was blunted with Tac-IR versus Tac-ER (Table 3).

Discussion

Our results suggest that renal perfusion is greater with the single dose, lower C_{max}, Tac-ER compared with the twice-daily Tac-IR. In addition, GFR also trended to be higher with Tac-ER.

In the United States, 85% and 72% of renal and liver transplant recipients, respectively, are on a tacrolimus-based immunosuppressive therapy at 1-year post-transplant [16,17]. The narrow toxic to therapeutic window of these agents requires close therapeutic drug monitoring for patients. The current trend in clinical practice has been toward safe minimization of CNI exposure, which has resulted in improved renal function and less acute rejection [18]. The deleterious effect of acute CsA exposure on renal hemodynamic function has been demonstrated by a number of investigators [1,4,5]. However, there are now compelling data demonstrating that effects on renal hemodynamic function are reduced with Tac-IR [6]. The decline in renal perfusion with CsA correlates with the peak blood concentration of this agent, suggesting a renal vasoconstrictive effect [1,3,5]. Recently, we have reported that compared with CsA, Tac-ER is associated with improved ERPF and GFR in healthy volunteers [11].

Given that Tac-ER has only a single dose exposure with a lower C_{max} compared with Tac-IR, we hypothesized that there might be an improvement in renal blood flow and GFR with Tac-ER versus Tac-IR. Using a model of intact renal function in healthy male subjects under carefully controlled physiological conditions, our first major observation was that Tac-ER was associated with a higher ERPF. Moreover, unlike our previous trial comparing Tac-ER to CsA over 6 h, in the current study, 24-h renal function was performed to adequately address the 24-h exposure of Tac-ER and the twice-daily 12-h dosing regime of Tac-IR. This

Table 3. Plasma aldosterone, 6-keto-prostaglandin F1α, and nitric oxide levels At the start of and 2 h following the start of each 24-h assessment. Values are mean ± SD. $n = 17$. *P*-values are based on paired *t*-test to compare the means of 0 h vs. 2 h within each group.

	Baseline		Tac-IR		Tac-ER	
	$T = 0$ h	$T = 2$ h	$T = 0$ h	$T = 2$ h	$T = 0$ h	$T = 2$ h
Serum Aldosterone (pmol/l)	160.3 ± 78.0	111.9 ± 64.7 ($P = 0.005$)	194.9 ± 129.5	123.1 ± 58.0 ($P = 0.020$)	171.1 ± 131.8	129 ± 87.9 ($P = 0.106$)
6-keto-Prostaglandin F1 α (pg/ml)	89.9 ± 54.7	65.7 ± 18.2 ($P = 0.048$)	105.4 ± 47.9	93.1 ± 51.9 ($P = 0.121$)	95.2 ± 52.3	66.1 ± 25.5 ($P = 0.007$)
Serum Nitric oxide (μmol/l)	54.2 ± 6.3	53.4 ± 7.9 ($P = 0.646$)	63.4 ± 10.2	58.2 ± 7.3 ($P = 0.002$)	61.9 ± 11.3	57.8 ± 11.0 ($P = 0.022$)

finding is also novel because it is to our knowledge, the first study involving human subjects whereby sinistrin clearance was performed over 24 h.

There were three observations in the current study: baseline (no Tac exposure), 10 days of Tac-ER and 10 days of Tac-IR exposure in a randomized cross-over design targeting a C_0 of 8–12 ng/ml. Although this target is at the higher level of early exposure in some clinical practices, we wanted to emulate real-life practice, particularly in extra-renal solid organ recipients where there may be additional renal insults in the early post-transplant period. Of importance was that by Day 7, trough tacrolimus concentrations were equivalent for both Tac-ER and Tac-IR although there was a delay in achieving steady state trough concentrations with Tac-ER, as has been previously described [8].

Using this regimen, our results support the hypothesis that renal perfusion is greater with the single dose, lower C_{max}, Tac-ER compared with the twice-daily Tac-IR. In addition, GFR also tended to be higher with Tac-ER although this did not achieve statistical significance. Somewhat less expected was the trend toward increased renal perfusion noted with Tac-ER compared with baseline. Although only speculative at this time, this may relate to enhanced reperfusion with Tac-ER on the basis of a slow release format. Nevertheless, the internal validity of the study was supported by a number of findings: (i) in all three study conditions, ERPF, RBF, and GFR all decreased during the nocturnal period. This is consistent with normal diurnal changes in renal hemodynamic function in healthy controls and in patients with underlying kidney disease [19–21]; (ii) as illustrated in Figs 3, 4, and 5, regardless of the tacrolimus formulation, following expected ERPF and GFR reductions at peak tacrolimus exposure at 1.5–2.5 h postdose, ERPF and GFR improved by 3–8 h.

Are there clinical data demonstrating improved renal function in patients receiving Tac-ER compared to Tac-IR? Two groups have previously reported improvements in renal function following conversion to Tac-ER in renal transplant recipients [22,23], although this may have been due to reduced tacrolimus exposure. Improvement in renal function was reported by Giannelli *et al.* [24] in a conversion study from Tac-IR to Tac-ER in 65 liver transplant recipients. Valente *et al.* [25] also reported similar findings in a smaller cohort of converted liver transplant recipients. In contrast, a multicenter conversion study of over 1800 renal transplant recipients did not demonstrate any renal function change following conversion [9]. Similarly, we did not demonstrate any change in eGFR in our single center conversion of 500 renal transplant recipients [26]. These seemingly conflicting previous literature may be due to the method used to assess renal function, as estimates of GFR in clinical practice are unlikely to be sufficiently sensitive or accurate to detect hemodynamic changes demonstrated in the present study under carefully controlled conditions using gold-standard tests of renal blood flow and GFR. In addition, the use of healthy volunteers with innervated kidneys may best reflect the renal hemodynamic effects of CNI exposure in the extra-renal solid organ transplant recipient better than the denervated solitary renal allograft.

Other investigators [27–29] have implicated neurohormones such as endothelin and renin as important regulators of renal hemodynamic responses to CNI, although this relationship is inconsistent [11]. In the present study, effects of Tac-IR and Tac-ER on nitric oxide and aldosterone did not differ. In contrast, plasma prostaglandin F1α declined less with Tac-IR versus Tac-ER. As prostaglandin F1α is generally considered to be an afferent renal arteriolar vasodilator, higher levels after Tac-IR would be expected to *increase* rather than reduce renal blood flow. As such, the mechanisms responsible for the higher 24-h renal perfusion remain unknown.

Our study has limitations. First, the sample size was small, which may have limited our ability to detect significant GFR differences. We attempted to minimize the impact of the small sample size by using a cross-over design allowing subjects to act as their own controls. Second, we recognize that it remains unclear if acute hemodynamic effects of CNIs are related to nephrotoxicity, including chronic renal pathological changes associated with long-term CNI use. Third, despite the recognition that chronic CNI nephrotoxicity is an important component of the renal failure in transplant recipients [2,30–33], we recognize that chronic CNI nephrotoxicity, may be evolving into a less common clinical entity [34–36]. We further acknowledge that the renal hemodynamic benefit associated with CNI withdrawal does not necessarily translate to improved renal histology [37]. Nevertheless, CNIs with superior PK profiles may be less nephrotoxic, such as with Tac-IR versus CsA [38–40]. For example, improved renal hemodynamic and PK profiles with low-dose Tac-IR in the 36-month follow-up SYMPHONY trial were associated with improved renal function and allograft survival compared with either the low-dose or standard CsA cohorts [18,41].

In summary, once-daily Tac-ER is associated with significant renal hemodynamic improvement compared to twice-daily dosed Tac-IR. Long-term studies looking at the impact of Tac-ER versus other CNIs on long-term nephrotoxicity are warranted.

Authorship

JZ and DZIC: researched data, wrote the manuscript. VL, MS and KM: contributed to statistical analyses, discussion, reviewed/edited manuscript. All authors have approved the final version of this manuscript.

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