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Therapeutic equivalence and pharmacokinetics of generic tacrolimus formulation in *de novo* kidney transplant patients

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ORIGINAL ARTICLE

ABSTRACT

Background. There is a growing concern about the therapeutic equivalence of the generic tacrolimus formulation (GEN Tacrolimus) to the reference tacrolimus (REF Tacrolimus) in solid organ transplantation.

Methods. A prospective, randomized study of 126 *de novo* renal transplant patients was conducted to compare the efficacy, safety and pharmacokinetic (PK) profiles between GEN tacrolimus ($n = 63$) and REF tacrolimus ($n = 63$). The PK of tacrolimus was evaluated on Day 10 and 6 months under steady-state condition. Crossover study was carried out in 66 patients at 6 months.

Results. On Day 10, 117 patients completed PK profiles (54 GEN tacrolimus and 63 REF tacrolimus) and GEN tacrolimus showed comparable C_0 (9.8 ± 2.5 versus 9.7 ± 3.0 ng/mL, P = 0.80) but significantly higher dose-normalized C_{max} (309.1 ± 191.9 versus 192.5 ± 95.2 ng/mL/mg/kg, P < 0.001). The dose-normalized AUC_{0-12} tended to be higher in the GEN tacrolimus than in the REF tacrolimus group (1513.4 ± 935.4 versus 1262.5 ± 593.5 ng.h/mL/mg/kg, P = 0.084). Because of this early and high C_{max} with a rapid decline in GEN tacrolimus concentration, the trough concentration was maintained lower than that of REF tacrolimus. At 6 months, GEN tacrolimus showed equivalent dose-normalized AUC_{0-12} (1882.2 ± 935.6 versus 1718.1 ± 946.3 ng.h/mL/mg/kg, P = 0.429) but still higher dose-normalized C_{max} (346.3 ± 184.4 versus 273.2 ± 148.9 ng/mL/mg/kg, P = 0.056), despite a reduced trough concentration (5.7 ± 1.6 versus 6.9 ± 2.2 ng/mL, P = 0.004). PK profiles evaluated at 9 months showed that generic substitution also resulted in an 'early and high C_{max}'. Efficacy and safety data were comparable over the 9-month study period.

Conclusions. Therapeutic equivalence and the PK of GEN tacrolimus should be evaluated in patients undergoing *de novo* renal transplantation.

INTRODUCTION

Tacrolimus is now available in different oral formulations around the world [1–9]. In the USA, the first generic tacrolimus formulation (GEN tacrolimus) was approved in 2009 by the US Food and Drug Administration and the preliminary results of generic substitution in stable transplant recipients

were recently reported [3, 10]. The GEN tacrolimus, Tacrobell® (Chong Kun Dang Pharmaceutical, Corp., Seoul, Korea) was approved in 2006 by the Korea Food and Drug Administration (KFDA), and the use of GEN tacrolimus is widespread in Korea [1, 11]. The GEN tacrolimus, Tacrobell®, will be available in Nigeria, Indonesia, Pakistan and Japan in 2013. Similar to the USA, the 90% confidence intervals (90% CI) for both C_{max} and AUC mean ratio of GEN tacrolimus should fall within the accepted limits of 0.8–1.25 in order to be considered bioequivalent in Korea. The manufacturer demonstrated bioequivance of GEN Tacrolimus (Tacrobell®) to a reference tacrolimus product (REF Tacrolimus) (Prograf®; Astellas Pharma, Tokyo, Japan) in healthy volunteers to gain approval by the KFDA (90% CIs for AUC and C_{max} 0.9328–1.2297 and 1.0467–1.2169, respectively).

However, because transplant patients are quite different from healthy volunteers in drug absorption, metabolism and secretion, a demonstration of bioequivalence by single-dose studies in healthy individuals cannot offer a sufficient guarantee of therapeutic equivalence in transplant patients [12–14]. In addition, tacrolimus has a narrow therapeutic index that requires therapeutic drug monitoring (TDM) to achieve a satisfactory balance between maximizing efficacy and minimizing serious dose-related toxicity, which are related with drug exposure [15, 16]. Tacrolimus therapy should be optimized by monitoring trough levels as a surrogate marker of drug exposure. However, generic manufacturers are not required to validate the branded drug's TDM strategy and the paucity of data currently makes it difficult to presume an equivalent relationship between drug levels and exposure [14].

Therefore, we investigated the therapeutic equivalence of GEN Tacrolimus (TacroBell®) to REF Tacrolimus (Prograf®) and compared pharmacokinetic (PK) parameters between the two formulations in *de novo* kidney transplant recipients to develop a TDM strategy for GEN Tacrolimus. We also evaluated steady-state PK parameters of the two formulations and the interchangeability of GEN Tacrolimus with REF Tacrolimus in a crossover extension study with a 1 : 1 switch.

MATERIALS AND METHODS

Patients

Adult patients (age, 18–65 years) with end-stage renal disease who were scheduled to receive a single-organ kidney transplant from either a live donor or a deceased donor with a compatible ABO blood type were enrolled. Exclusion criteria included multiple organ transplant or previous nonrenal transplant, uncontrolled infections including HIV, significant liver disease, severe gastrointestinal disorders, pregnant or breast-feeding, a kidney graft from donors after cardiac death or a history of malignancy within 5 years.

This study (ClinicalTrials.gov NCT01055964) was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and the International Conference on Harmonization guidelines as well as Declaration of Istanbul. This study protocol was approved by the Seoul National University Hospital Institutional Review Board (IRB No H-0805-032-242), and patients provided written informed consent before study enrollment.

Study design

This 6-month, open-label, prospective, randomized, single-center study was conducted at the Seoul National University Hospital. Patients were randomly assigned in a 1 : 1 ratio to one of the study groups: the GEN tacrolimus group, which received GEN tacrolimus (TacroBell®); and the REF tacrolimus group, which received an innovator tacrolimus product (Prograf®). A random number table was used to allocate the participants.

After completion of the 6-month study period, patients who gave consent to a further crossover study underwent a drug switch from the reference to generic product or from the generic to the reference product based on their study group. A 1 : 1 dose conversion was employed at the time of the switch, and the tacrolimus dose was then adjusted to maintain predefined target trough levels.

Enrolled patients were asked to fast for at least 2 h before receiving tacrolimus and to take tacrolimus 1 h before or 2 h after a meal. On the day of the PK study, the same food was served for lunch. Patients received the same doses of tacrolimus for at least 2 weeks prior to the PK studies at 6 and 9 months to attain steady-state condition.

Immunosuppression

Both groups received induction antibody therapy with basiliximab and triple maintenance immunosuppressive therapy including tacrolimus, mycophenolate mofetil and steroids. The first doses of tacrolimus and mycophenolate mofetil were administered within 24 h before transplantation. After an initial oral dose of tacrolimus of 0.16 mg/kg/day divided into two daily doses, the tacrolimus dose was adjusted to achieve target trough levels according to the daily TDM in both groups. The oral form of both tacrolimus products was used throughout the study. Whole-blood target trough concentrations were 8–12 ng/mL for up to 3 months, 6–8 ng/mL until 6 months and 4–6 ng/mL thereafter. Mycophenolate mofetil was given at an initial dose of 1 g/day. Methylprednisolone was administered as 500 mg intravenous bolus dose at the time of surgery and was tapered gradually to a maintenance dose of 5 mg by 1 month after transplantation.

Monitoring

Tacrolimus trough levels were determined, using high-performance liquid chromatography tandem mass spectroscopy (HPLC/MS/MS) [17]. The intraday coefficient of variation (CV) ranged from 5.2 to 9.3% and the accuracy was 96.0–104.0%. The interday CV varied from 3.6 to 9.6%. The lower limit of quantitation for tacrolimus was 0.8 ng/mL.

In patients who participated in the additional crossover study, tacrolimus trough levels were measured weekly for 2 weeks after the drug switch, and once per month until 9 months after transplantation.

Routine kidney biopsies were performed at implantation and Day 10 in all patients, and a clinically indicated biopsy was performed on all suspected acute rejections prior to

initiating antirejection therapy. The biopsies were evaluated by a single pathologist following the Banff 97 criteria and updates [18, 19].

PK analysis

Tacrolimus PK was evaluated on Day 10 and 6 months after transplantation in both groups and 9 months after transplantation in patients who participated in the crossover study, by sampling peripheral whole-blood samples just before and at 0.5, 1, 2, 4, 6, 8 and 12 h after the morning dose. The trough level, as the lowest concentration immediately before tacrolimus administration (C_0), peak tacrolimus concentration (C_{max}) and the time required to reach C_{max} (T_{max}) for each subject were obtained directly from the raw data. The AUC_{0-12} was calculated using linear trapezoidal rules from Hours 0–12.

Objectives and end points

The objective of this study was to compare the PK parameters of tacrolimus during the early period after tacrolimus administration and those under steady-state conditions after transplantation in *de novo* kidney transplant recipients taking GEN tacrolimus or REF tacrolimus, and to evaluate the interchangeability between the two tacrolimus formulations in stable kidney transplant recipients.

The primary end-point was a comparison of systemic exposure (AUC_{0-12}) between the GEN tacrolimus and the REF tacrolimus groups on Day 10 and 6 months after transplantation in the PK evaluation set.

Secondary end points included renal function as indicated by the estimated glomerular filtration rate (eGFR) with the Modification of Diet in Renal Disease (MDRD) equation [20] during the course of the study, patient survival, allograft survival at 6 months, the biopsy-proven acute rejection (BPAR) event rate within 6 months following transplantation, the incidence of patient-reported adverse events (AEs) and all AEs including biochemical and hematological assessments. PK parameters at 6 and 9 months, and tacrolimus intraindividual variability (IIV) were also compared in acrossover extension study group. Tacrolimus levels between 3 and 9 months after transplantation were used to calculate and compare IIV between the two formulations. Tacrolimus IIV was calculated using a formula described previously [21].

Statistical analysis

Because of the absence of a reference for the GEN tacrolimus PK variables in renal transplant patients, 80 patients (40 per group) were the target number for outcome assessment, which is the number usually required for a standard bioequivalence trial.

The groups of patients who underwent analysis included a PK evaluation set (patients undergoing a PK evaluation on both Day 10 and 6 months) and an intention-to-treat population (patients who received at least one dose of study drug). The safety analysis was based on the intention-to-treat population. Unless stated, all other results including the PK parameters were based on the PK evaluation set.

In the crossover study, statistical comparisons of tacrolimus exposure and C_{max} at steady state were performed using a 90% CI approach. A 90% CI was constructed for the difference in mean natural log-transformed dose-normalized data between the GEN tacrolimus and REF tacrolimus. The CI was transformed back to the original scale and compared with an 80–125% range to determine the equivalence of tacrolimus exposure. Statistical analyses were conducted using SPSS software version 17.0 (SPSS, Inc., Chicago, IL, USA). All tests were two tailed, and P-values <0.05 were considered significant.

RESULTS

Patients

From December 2008 to January 2011, 126 patients were enrolled and randomized. A total of 117 patients (54 GEN Tacrolimus; 63 REF tacrolimus) completed at least 10 days of the PK study (Table 1). In total, 39.7% of the GEN Tacrolimus group and 12.7% of the REF tacrolimus group withdrew (Figure 1, P = 0.001). Thus, 93 patients completed the 6-month study (38 GEN tacrolimus; 55 REF tacrolimus). Both groups were well balanced with respect to demographic, immunological and donor–recipient characteristics, with no significant differences between the groups were observed (Table 2). No patients received nonimmunosuppressive drugs that interacted with tacrolimus. There were no gastrointestinal disorders that interfered with tacrolimus absorption present in any patient at the time of the PK study. Sixty-nine patients gave consent to participate in the crossover extension study and converted tacrolimus based on a mg : mg switch. Two patients withdrew consent, and one patient was dropped because of graft failure. Thus, 66 patients completed the 9-month crossover extension study (Figure 1).

PK analysis

The mean blood concentration–time profiles of the two tacrolimus formulations are shown in Figure 2. At 10 days after transplantation, GEN tacrolimus was associated with higher dose-normalized C_{max} (309.1 ± 191.9 versus 192.5 ± 95.2 ng/mL/mg/kg, P < 0.001), shorter time to C_{max} (T_{max}) (1.0 ± 0.5 versus 1.4 ± 0.8 h, P = 0.002) and slightly higher dose-normalized AUC_{0-12} (1513.4 ± 935.4 versus 1262.5 ± 593.5 ng.h/mL/mg/kg, P = 0.084), with comparable C_0 (9.8 ± 2.5 versus 9.7 ± 3.0 ng/mL, P = 0.803) and weight-normalized dose (0.14 ± 0.08 versus 0.13 ± 0.05 mg/kg, P = 0.447). Because of this 'early and high C_{max}' PK pattern and concern for calcineurin inhibitor (CNI) nephrotoxicity, the doses of patients allocated to the GEN tacrolimus group were reduced. Consequently, C_0 (5.7 ± 1.6 versus 6.9 ± 2.2 ng/mL, P = 0.004) and weight-normalized dose (0.069 ± 0.03 versus 0.086 ± 0.04 mg/kg, P = 0.04) were significantly lower in the GEN tacrolimus group and this resulted in a comparable dose-normalized AUC_{0-12} (1882.2 ± 935.6 versus 1718.1 ± 946.3 ng.h/mL/mg/kg, P = 0.429) despite a still higher dose-normalized C_{max} (346.3 ± 184.4 versus 273.2 ± 148.9 ng/mL/mg/kg, P = 0.056) in the GEN tacrolimus group at the 6-month PK analysis.

There was a different correlation between C_0 and AUC_{0-12} for REF tacrolimus and GEN tacrolimus and the slope of the

Table 1. Baseline characteristics of the patients who completed at least the 10-day pharmacokinetic evaluation

	Generic tacrolimus group ($n = 54$)	Reference tacrolimus group ($n = 63$)	P-value
Sex (M:F)	33 : 21	37 : 26	0.85
Age (years)	45.8 ± 12.1	46.1 ± 12.5	0.90
Donor age (years)	43.0 ± 12.9	42.1 ± 13.8	0.73
Body mass index	21.3 ± 2.4	22.6 ± 3.2	0.02
Donor type (Live:Deceased)	26 : 28	28 : 35	0.58
Retransplantation (%)	3 (5.8)	6 (9.4)	0.47
HLA Ag mismatches	3.54 ± 1.69	3.37 ± 1.60	0.59
PRA > 20% (*n*)	7 (13.5)	7(10.9)	0.78
Delayed graft function, *n* (%)	4 (7.4)	8 (12.7)	0.38
Concomitant immunosuppressant dosage (mean) at Day 10			
MMF dose (g/day)	1.0 ± 0.2	1.1 ± 0.3	0.12
Prednisone dose (mg/day)	10.7 ± 3.6	10.8 ± 3.6	0.85
Laboratory findings at Day 10			
Hemoglobin (g/dL)	10.5 ± 1.3	10.5 ± 1.6	0.82
Albumin (mg/dL)	3.54 ± 0.35	3.48 ± 0.32	0.39
Serum creatinine (mg/dL)	1.73 ± 1.81	1.34 ± 0.93	0.16

PRA, panel-reactive antibody; MMF, mycophenolate mofetail.

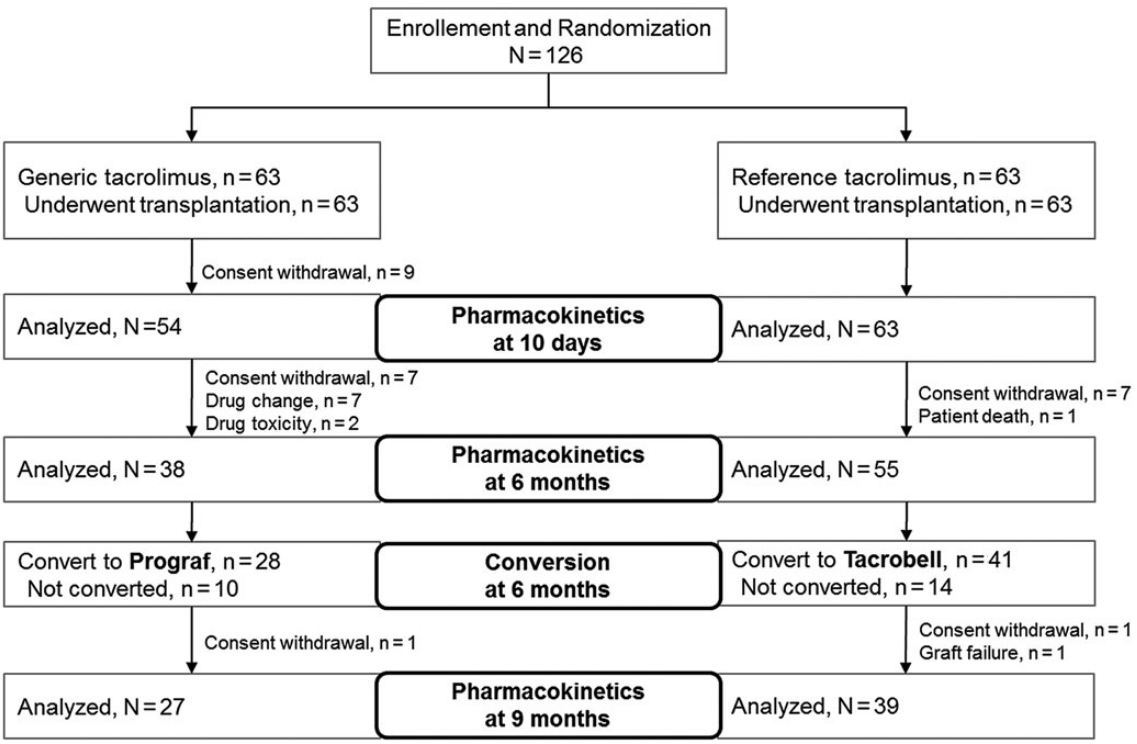
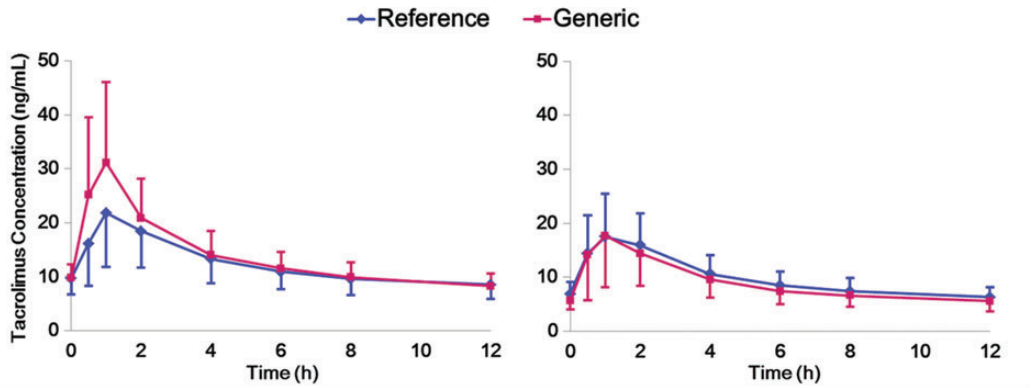


FIGURE 1: Patient disposition (CONSORT diagram).

Table 2. Baseline characteristics of the patients who completed both 10-day and 6-months pharmacokinetic evaluations

	Generic Tacrolimus group (n = 38)	Reference Tacrolimus group (n = 55)	P-value
Sex (M:F)	23 : 15	31 : 24	0.83
Age (years)	47.0 ± 12.7	45.6 ± 12.4	0.62
Donor age (years)	44.0 ± 13.3	41.1 ± 12.6	0.31
Body mass index	21.5 ± 2.3	22.8 ± 3.3	0.04
Donor type (Live:Deceased)	15 : 23	23 : 32	0.83
Retransplantation (%)	2 (5.3)	6 (10.9)	0.47
HLA Ag mismatches	3.47 ± 1.70	3.35 ± 1.53	0.73
PRA > 20% (n)	5 (13.2)	5 (9.1)	0.74
Delayed graft function, n (%)	4 (10.5)	8 (14.6)	0.38
Concomitant immunosuppressant dosage (mean) at Month 6			
MMF dose (g/day)	1.0 ± 0.5	1.0 ± 0.4	0.40
Prednisone dose (mg/day)	5.0 ± 0.0	5 ± 0.0	0.85
Laboratory findings at Month 6			
Hemoglobin (g/dL)	12.6 ± 1.3	12.3 ± 0.5	0.57
Albumin (mg/dL)	4.35 ± 0.28	4.34 ± 0.31	0.85
Serum creatinine (mg/dL)	1.21 ± 0.32	1.22 ± 0.25	0.89

PRA, panel-reactive antibody; MMF, mycophenolate mofetil.



	Day 10			Month 6		
	Reference (n=63)	Generic (n=54)	P-value	Reference (n=55)	Generic (n=38)	P-value
C_0 (ng/mL)	9.7±3.0	9.8±2.5	0.803	6.89±2.2	5.65±1.6	0.004
C_{max} (ng/mL)	23.4±9.1	35.1±14.5	<0.001	19.6±7.4	19.6±9.5	0.989
T_{max} (h)	1.4±0.8	1.0±0.5	0.002	1.54±1.11	1.31±0.87	0.309
AUC_{0-12} (ng.h/mL)	147.9±43.8	164.0±44.4	0.051	118.5±34.2	106.8±34.7	0.111
Dose/weight (mg/Kg)	0.13±0.05	0.14±0.08	0.447	0.086±0.04	0.069±0.03	0.040
Dose normalized C_0 (ng/mL/mg/Kg)	85.1±44.3	95.8±62.7	0.290	102.6±62.5	100.6±51.1	0.870
Dose normalized C_{max} (ng/mL/mg/Kg)	192.5±95.2	309.1±191.9	<0.001	273.2±148.9	346.3±184.4	0.056
Dose normalized AUC_{0-12} (ng.h/mL/mg/Kg)	1262.5±593.5	1513.4±935.4	0.084	1718.1±946.3	1882.2±935.6	0.429

FIGURE 2: Pharmacokinetic parameters on Day 10 (left panel) and at Month 6 (right panel).

line of best fit was different for both formulations (Figure 3). On Day 10, REF tacrolimus showed a stronger correlation ($r = 0.653$) and more acute slope (unstandardized coefficient = 9.56) than GEN tacrolimus ($r = 0.420$, unstandardized coefficient = 7.486). At 6 months after transplantation, on the other hand, GEN tacrolimus had a stronger correlation ($r = 0.824$) and more acute slope (unstandardized coefficient = 17.865) than REF tacrolimus ($r = 0.727$, unstandardized coefficient = 11.283). The equivalence of tacrolimus exposure was not demonstrated based on $\ln AUC_{0-12}$ at Day 10 and 6 months after transplantation (Table 3); the ratio of $\ln AUC_{0-12}$ for GEN tacrolimus/REF tacrolimus were 105.6 and 109.8, respectively, for Day 10 and 6 months, and the respective 90% CIs were 88.3–126.3% and 93.0–138.0%.

Efficacy and safety over the 6-month study period

The patient and graft survival rates were comparable between the two tacrolimus groups during the 6-month study. One patient receiving REF tacrolimus died due to intracranial hemorrhage. No death or graft loss was observed in the GEN tacrolimus group. Renal function was similar between the two treatment groups throughout the 6-month study period. At 6 months after transplantation, $eGFR_{MDRD}$ was 66.3 ± 18.5 and 64.4 ± 16.7 mL/min for the REF tacrolimus and GEN tacrolimus groups, respectively (P = 0.585). The frequencies of clinical BPAR were 4.8 and 3.7% (P = 0.850), and the subclinical acute rejection in protocol biopsies were more frequent in the GEN tacrolimus group (14.8%) than the REF tacrolimus group (3.2%, P = 0.043). All BPARs including both clinical and subclinical ARs were treated with steroid pulse.

No marked differences in the incidence or severity of patient-reported AEs between the REF tacrolimus and the GEN tacrolimus groups (Table 4). However, two patients in the GEN tacrolimus group showed drug-related laboratory abnormalities such as elevated liver enzymes 4-fold greater than the normal upper limit and renal dysfunction related with elevated tacrolimus trough levels.

Crossover study subgroup analysis

Sixty-six patients participated in the crossover extension study (39 REF tacrolimus, 27 GEN tacrolimus), and the baseline characteristics were comparable between the two subgroups (Table 5). No patient in either group experienced AEs such as acute rejection or laboratory abnormalities after conversion and showed comparable renal graft function during the crossover extension study period (data not shown).

Twenty-two patients (56.4%) in the REF tacrolimus group underwent dose reduction after conversion to GEN tacrolimus; only one patient (2.6%) required a dose increase to maintain the AUC_{0-12}; mean weight-normalized dose decreased from 0.086 ± 0.039 mg/kg of the REF tacrolimus at 6 months to 0.075 ± 0.039 mg/kg of the GEN tacrolimus at 9 months (P < 0.001).

The converted subgroup underwent an additional PK evaluation at 9 months after transplantation, which was under steady-state conditions after the switch in tacrolimus formulations (Figure 4). Generic substitution of tacrolimus resulted in an 'early and high C_{max} with rapid wash-out' pattern in the AUC curve and showed a higher dose-normalized AUC_{0-12} (1896.9 ± 1000.6 versus 1641.1 ± 849.8 ng.h/mL/mg/kg, P = 0.023) and C_{max} (343.8 ± 154.1 versus 268.7 ± 150.8 ng/mL/mg/kg, P = 0.014) at 9 months, although C_0 and weight-normalized tacrolimus dose (0.075 ± 0.039 versus 0.086 ± 0.039 mg/kg, P < 0.001) were maintained at lower levels. The geometric mean ratio (GEN to REF tacrolimus) of dose-normalized $\ln AUC_{0-12}$ and ln C_{max} were 109.9 and 123.9, and the respective 90% CIs for these were 94.2–128.3% and 105.8–145.1% in the crossover study (Table 3). The mean IIV of tacrolimus was 13.6 ± 7.3% in the REF tacrolimus group before the drug switch and 14.2 ± 6.8% after the switch to GEN tacrolimus (P = 0.725).

DISCUSSION

Licensing a generic drug usually requires a demonstration of pharmaceutical equivalence and bioequivalence with the reference product, which does not require preclinical or clinical data to establish efficacy and safety data [22]. A big controversy surrounding GEN tacrolimus currently exists; that is, whether a demonstration of bioequivalence by a single fixed dose study in 20 healthy adults can offer a sufficient guarantee of therapeutic equivalence in solid organ transplant patients [14]. There is little evidence indicating whether the TDM strategy of the brand-new tacrolimus can be applied to patients treated with GEN tacrolimus. Therefore, the American Society of Transplantation and the American Society of Transplant Surgeons emphasize that bioequivalence should be demonstrated in an at-risk population during the drug approval process and that bioequivalence testing of generic immunosuppressive products is essential in transplant recipients [23, 24].

No well-designed randomized clinical trials are available, which have compared the therapeutic equivalence of GEN tacrolimus to REF tacrolimus in renal transplant recipients. Three groups including ours have shown acceptable short-term outcomes with generic formulations in single-arm, nonrandomized clinical studies with *de novo* kidney transplant patients [4, 25, 26], and a few nonrandomized conversion trials have been reported [3]. To the best of knowledge, this study is the first randomized, controlled study comparing bioequivalence and therapeutic equivalence of the GEN tacrolimus to REF tacrolimus in *de novo* kidney transplant patients.

The equivalence of tacrolimus exposure was not demonstrated at Day 10, month 6 and in crossover study subgroup (Table 3). The ranges of confidence intervals did not meet the accepted limits of 80–125% (US FDA) or 90.0–111.1% (European Medical Agency (EMA)) in this intended patient population in spite of acceptable margin (US and Korea FDAs) of AUC (0.9328–1.2297) and C_{max} (1.0467–1.2169) in healthy volunteers. This highlights that narrow therapeutic index drugs are required to meet a stricter acceptance interval for AUC and C_{max} in a standard bioequivalence study such as EMA criteria. Although our study showed comparable clinical outcomes with GEN tacrolimus in *de novo* kidney transplant

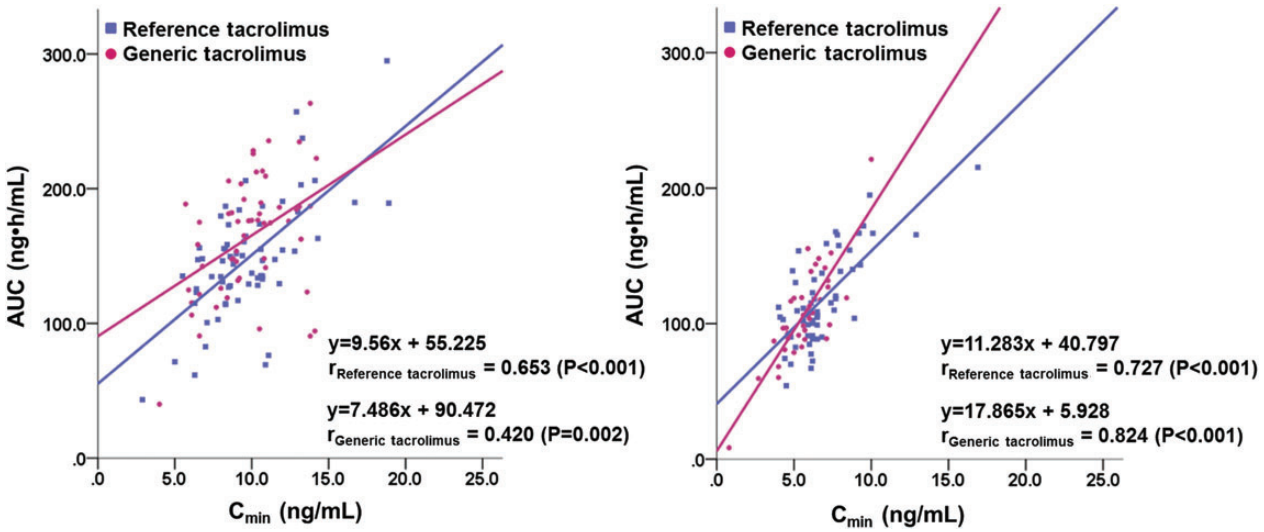


FIGURE 3: Correlation between C_{min} and AUC_{0-12} for reference tacrolimus and generic tacrolimus on Day 10 (left panel) and Month 6 (right panel).

Table 3. Equivalence of tacrolimus exposure

Dose-normalized	GEN tacrolimus mean	REF Tacrolimus mean	Ratio (GEN/REF)	90% CI
Day 10				
ln(AUC_{0-12})	26.8	23.5	105.6	88.3–126.3
ln(C_{max})	5.5	3.6	142.5	118.7–171.1
Month 6				
ln(AUC_{0-12})	33.8	29.9	109.8	93.0–138.0
ln(C_{max})	6.2	4.8	114.5	101.2–152.3
Crossover				
ln(AUC_{0-12})	33.2	30.0	109.9	94.2–128.3
ln(C_{max})	6.1	4.9	123.9	105.8–145.1

GEN tacrolimus, generic tacrolimus formulation; REF tacrolimus, reference tacrolimus formulation; CI, confidence interval.
Natural log (ln) parameter means, ratios, and confidence intervals were calculated by transforming the natural log-dose-normalized means back to the linear scales.

patients during the study period, in the clinical perspectives, the long-term transplant outcome including tacrolimus side effects is certainly worth investigating.

We evaluated the PK characteristics of a GEN tacrolimus formulation (Tacrobell®) in renal transplant patients. The mean blood concentration–time profiles (Figure 2) during the early period showed 'early and high peak concentration of tacrolimus and a resulting high AUC_{0-12}' in the GEN tacrolimus group despite comparable weight-normalized dose and C_0. This may have been caused by the high dissolution rate of GEN tacrolimus and resulting rapid uptake as shown in an *in vitro* study [27]. The high peak tacrolimus concentration demonstrated in the 10-day PK evaluation raised concerns about CNI nephrotoxicity, and the tacrolimus dose was decreased in the GEN tacrolimus group. Systemic exposure of tacrolimus, measured as dose-normalized AUC_{0-12}, was comparable at 6 months. Therefore, GEN tacrolimus (Tacrobell®) should be prescribed at a slightly lower dose than the brand product, and the C_0 should be maintained 15% lower to maintain peak concentration that is not too high along with an appropriate AUC.

Interchangeability between REF tacrolimus and GEN Tacrolimus is also an important issue in stable renal transplant recipients. We reduced the tacrolimus dose in more than half of the patients, and mean tacrolimus trough level declined from 6.6 ± 1.9 to 6.0 ± 1.9 ng/mL ($P = 0.089$) to maintain equivalent systemic tacrolimus exposure after conversion to GEN tacrolimus in REF tacrolimus group. This means that generic substitution of tacrolimus with the Tacrobell®-generic formulation requires a dose reduction to decrease trough levels

by 10%. Therefore, this result suggests that changes in the tacrolimus formulation in renal transplant patients should be carried out by clinicians who are familiar with the PK characteristics of both tacrolimus products and that this is followed with heightened TDM.

Table 4. Reported adverse events within 6 months post-transplantation

	Reference tacrolimus group	Generic tacrolimus group	P-value
Drug-related laboratory abnormality[a]	0 (0.0)	2 (3.7)	0.211
Nephrotoxicity[b]	3 (4.8)	2 (3.7)	1.00
Infection, *n* (%)	10 (15.9)	8 (14.8)	1.00
PTDM, *n* (%)	1 (1.6)	2 (3.7)	0.595
Hair loss, *n* (%)	5 (7.9)	6 (11.1)	0.752
Hand tremor, *n* (%)	4 (6.4)	2 (3.7)	0.685
Headache, *n* (%)	2 (3.2)	1 (1.9)	1.00
Psychiatric, *n* (%)	0 (0.0)	2 (3.7)	0.211

Drug-related laboratory abnormalities[a] includes elevation of hepatic enzyme and serum creatinine. Nephrotoxicity[b] is identified by all biopsies after transplantation. PTDM, posttransplant diabetes mellitus.

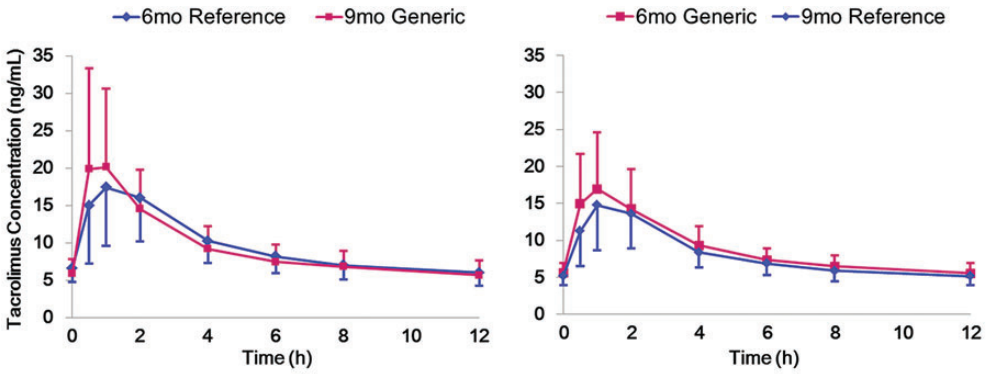
The current study had several limitations. First, the study population was relatively small, showed high discontinuation rate, and most of the patients were Asian. Second, factors involved in interindividual variability such as a genetic polymorphism of *CYP3A5* and *ABCB1* were not assessed and should be investigated further. Last, a supplementary pharmacoeconomic study should be carried out because the purpose of generic substitution is to provide a less-expensive drug to save cost. However, this study had several strengths. First, HPLC/MS/MS assay was used to determine tacrolimus concentrations, which ensures an accurate measure of systemic exposure [28]. Second, PK parameters were evaluated twice. Finally, the crossover design of the extension study enhanced the understanding of the differences in PK between the two formulations.

In summary, immunosuppression using GEN tacrolimus (Tacrobell®) resulted in different PK parameters such as earlier and higher peak tacrolimus concentrations both during the early period and under steady-state conditions compared with those of the REF product in *de novo* kidney transplant recipients. Switching from REF tacrolimus to the GEN formulation (Tacrobell®) required a small but significant dose reduction in stable kidney transplant recipients. Despite this finding, GEN tacrolimus (Tacrobell®) can be safely used in *de novo* renal transplant patients and transplant patients currently taking REF tacrolimus can be safely switched to the generic formulation provided that transplant clinicians are familiar with the PK characteristics of the two tacrolimus formulations and the heightened TDM is carried out.

Table 5. Demographics of crossover study subgroup

	Generic tacrolimus group (*n* = 27)	Reference tacrolimus group (*n* = 39)	P-value
Sex (M:F)	16 : 11	25 : 14	0.798
Age (years)	47.9 ± 12.9	44.1 ± 12.4	0.228
Donor age (years)	43.5 ± 13.0	41.3 ± 12.1	0.492
Body mass index	21.3 ± 2.5	22.9 ± 3.2	0.030
Donor type (live:deceased)	8 : 19	14 : 25	0.791
Retransplantationn (%)	1(3.7)	5 (12.8)	0.388
HLA Ag mismatches	3.23 ± 1.82	3.31 ± 1.61	0.862
PRA > 20% (*n*)	7 (25.9)	5 (12.8)	1.00
eGFR at conversion	62.32 ± 15.4	64.3 ± 15.3	0.613
Concomitant immunosuppressant dosage (mean) at Month 6			
MMF dose (g/day)	0.9 ± 0.5	1.0 ± 0.4	0.74
Prednisone dose (mg/day)	4.5 ± 1.8	4.4 ± 1.6	0.33
Laboratory findings at Month 6			
Hemoglobin (g/dL)	12.3 ± 0.5	12.6 ± 1.3	0.57
Albumin (mg/dL)	4.35 ± 0.31	4.36 ± 0.32	0.89

BMI, body mass index; PRA, panel-reactive antibody; eGFR; estimated glomerular filtration rate.



	Conversion from reference to generic			Conversion from generic to reference		
	6 month (n = 39)	9 month (n = 39)	P-value	6 month (n = 27)	9 month (n = 27)	P-value
C_0 (ng/mL)	6.6±1.9	6.0±1.9	0.089	5.6±1.3	5.2±1.3	0.182
C_{max} (ng/mL)	19.7±7.4	23.1±12.2	0.112	18.8±7.6	15.6±5.7	0.025
T_{max} (h)	1.44±1.1	1.18±1.1	0.322	1.1±0.6	1.4±0.8	0.096
AUC_{0-12} (ng.h/mL)	116.3±32.4	112.5±35.4	0.580	105.5±25.3	95.8±24.4	0.129
Dose/weight (mg/Kg)	0.086±0.039	0.075±0.039	<0.001	0.066±0.034	0.063±0.037	0.231
Dose-normalized C_0 (ng/mL/mg/Kg)	95.5±53.3	105.7±65.6	0.083	109.0±57.4	101.8±48.5	0.340
Dose-normalized C_{max} (ng/mL/mg/Kg)	268.7±150.8	343.8±154.1	0.014	358.7±201.6	296.6±139.1	0.033
Dose-normalized AUC_{0-12} (ng.h/mL/mg/Kg)	1641.1±849.8	1896.9±1000.6	0.023	2031.0±992.7	1872.5±795.5	0.184

FIGURE 4: Changes in pharmacokinetic parameters in the crossover extension study subgroup. Conversion from the reference product to the generic formulation (left panel) versus conversion from the generic formulation to the reference product (right panel).

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CONFLICT OF INTEREST STATEMENT

None declared.

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ORIGINAL ARTICLE

HLA-A2, HLA-B44 and HLA-DR15 are associated with lower risk of BK viremia

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ABSTRACT

Background. Human leucocyte antigens (HLAs) modulate immunity to polyomavirus BK (BKV). Identification of HLAs that alter the course of infection will facilitate risk stratification, and customization of pre-emptive intervention strategies.
Methods. We performed a retrospective cohort study with 998 kidney transplant patients with BKV infection status confirmed by polymerase chain reaction (PCR). Clinical parameters and donor–recipient matching for specific HLAs were examined in relation to occurrence of viremia. An emphasis was placed on donor–recipient matching rather than the actual frequency of specific HLA-alleles, since a successful immune response requires sharing of HLAs between a virus-infected target cell and the anti-viral effector cell.
Results. Using multivariate statistics, low risk of BK viremia was associated with matching of HLA-A2 [hazard ratio (HR) 0.51, 95% confidence interval (CI) 0.28–0.85], HLA-B44 (HR 0.31, 95% CI 0.076–0.85) and HLA-DR15 (HR 0.35, 95% CI 0.084–0.93) ($P < 0.05$), whereas high risk of viremia was