Impact of CYP3A5 Genetic Polymorphism on Intrapatient Variability of Tacrolimus Exposure in Chinese Kidney Transplant Recipients

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ABSTRACT

Background. Although high tacrolimus (FK) intrapatient variability (IPV) was shown to be associated with poor graft outcome in kidney transplant recipients (KTRs), it is uncertain whether there is any association between the CYP3A5 genotype and IPV of FK concentrations. Instead of trough level, we use calculated abbreviated AUC_{0-12} to investigate the impact of CYP3A5 genetic polymorphism on IPV of FK pharmacokinetics.

Methods. We conducted a retrospective, single-center study of 86 adult Chinese KTRs with known CYP3A5 genotype. Coefficient of variation (CV) was used for the quantification of FK IPV. CV of dose-normalized FK AUC_{0-12} was calculated and was compared between the CYP3A5 expresser group and nonexpresser group.

Results. Forty-one patients (47.7%) were classified as CYP3A5 expressers while 45 were nonexpressers. No significant differences in the baseline characteristics were found between expressers and nonexpressers. CYP3A5 expressers required 1.8 times higher FK dose compared with the nonexpressers. There was no significant difference in the FK CV between CYP3A5 expressers (18.2 ± 7.5%) and nonexpressers (16.7 ± 5.7%) ($P = .31$).

Conclusion. The IPV of FK exposure was not associated with CYP3A5 genotype in stable KTRs. Further studies should focus on other factors such as medication nonadherence, which may explain FK IPV.

TACROLIMUS (FK) is a calcineurin inhibitor used worldwide for primary immunosuppression following kidney transplantation. However, because of its narrow therapeutic window and variable pharmacokinetics, FK blood concentrations must be closely monitored. FK is metabolized by the cytochrome P450 3A (CYP 3A) enzymes and transported in the gut by P-glycoprotein, which is encoded by the ABCB1 gene. CYP3A5 exhibits variable expression in different individuals; one can be either an "expresser" of functional CYP3A5 (presence of at least 1 wild-type *1 allele, denoted as *1/*1 and *1/*3) or a "nonexpresser" (homozygous for the mutant *3 allele, denoted as *3/*3). Previous studies showed that CYP3A5 expressers required a higher FK dose to achieve targeted concentrations than CYP3A5 nonexpressers [1,2].

In contrast to the large amount of studies investigating the interpatient variability in FK pharmacokinetics, relatively few studies focused on the issue about intrapatient variability (IPV). Borra et al showed that a high degree of IPV in FK trough level was associated with reduced allograft survival [3]. In addition, high IPV was found to be related to accelerated progression of chronic histologic lesions before any evidence of renal dysfunction. A recent study also found that IPV of FK trough level was strongly associated with acute rejection in African Americans [4]. This IPV has been demonstrated to be strongly related to medication compliance, although it may have other causes including concomitant medication, diet, changing hematocrit, liver dysfunction, and diarrhea [5].

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Korean investigators found that the CYP3A5*3/*3 genotype had over 50% greater IPV of FK concentrations than the CYP3A5*1/*1 and *1/*3 group [6]. However, a similar relationship between the CYP3A5 genotype and FK IPV was not found in other studies [7,8].

It is well recognized that CYP3A5 allele frequency differs between ethnic groups. The CYP3A5*3 polymorphism occurred homozygously in 90% of the Caucasians, 53% of the Chinese, and 30% of the African Americans [2,9,10]. Most of the previous studies correlating CYP3A5 genotype with FK IPV were focused on the Caucasian population, and there is a paucity of such data in other races, especially Chinese patients who have a high prevalence of CYP3A5*1 allele. In addition, our previous study demonstrated that abbreviated 12-hour area under the curve ($AUC_{0\text{-}12}$) obtained by 2-time point regression equation using 2-hour (C_2) and 4-hour (C_4) FK concentrations is a better surrogate marker than trough level (C_0) for drug exposure in Chinese kidney transplant recipients (KTRs) [11]. Therefore, we investigate the frequency of IPV of FK concentrations in the Chinese KTRs and whether CYP3A5 genotype is related to IPV of FK abbreviated $AUC_{0\text{-}12}$ in those patients.

MATERIAL AND METHODS

This was a retrospective, single-center study of all Chinese KTRs aged 18 years or above who received twice-daily FK (Prograf) as part of the immunosuppressive regimen and were genotyped for CYP3A5*3 single-nucleotide polymorphism during a pharmacogenetic study conducted by our group [2]. All these patients had regular follow-ups in Queen Elizabeth Hospital, Hong Kong. They were divided into 2 groups; those with at least 1 wild-type allele (CYP3A5*1) express CYP3A5 are expressers while homozygotes for the mutant allele CYP3A5*3 are nonexpressers.

In our center, FK blood concentrations were determined 2 (C_2) and 4 (C_4) hours after the morning dose of FK administration in EDTA whole blood using a semi-automated microparticle enzyme immunoassay on an Imx II clinical analyzer (Abbott Laboratories, Abbott Park, Ill, United States). These 2 FK blood concentrations determined were used to calculate the $AUC_{0\text{-}12}$ according to the equation described by our group: $AUC_{0\text{-}12} = 16.2 + 2.4 \times C_2 + 5.9 \times C_4$. The daily FK dose was then adjusted according to the $AUC_{0\text{-}12}$ value, which was kept at around 100 to 150 ng×h/mL in the first 3 months. After 3 months, the target $AUC_{0\text{-}12}$ value was decreased to around 80 to 100 ng×h/mL for long-term maintenance. These $AUC_{0\text{-}12}$ values were based on our previous pilot study and clinical experience [11]. For each patient, at least 3 FK $AUC_{0\text{-}12}$ and the corresponding FK doses dated 6 months to 18 months after transplantation were collected. There was no change in the daily FK dose for at least 2 weeks, and none of the patients included were taking medication known to have interaction with FK. Our patients were instructed to take FK under fasting conditions, that is, 1 hour before and 2 hours after a meal. All basic demographic and clinical parameters were extracted from the patients' electronic medical record. The coefficient of variation (CV) can be used for the quantification of IPV in FK concentrations [5,12]. The whole-blood FK $AUC_{0\text{-}12}$ were corrected for the drug dosage by dividing the abbreviated $AUC_{0\text{-}12}$ by the daily dose. In our study, CV (%) of dose-normalized (dn) FK $AUC_{0\text{-}12}$ was calculated using the formula: (standard deviation $dnAUC_{0\text{-}12}$/mean $dnAUC_{0\text{-}12}$) × 100%. The CV was compared between the CYP3A5 expresser group and nonexpresser group. In addition, patients were also then divided into low- and high-CV groups using the median CV for each of the 2 groups as the cutoff value, the approach used by Borra et al [3]. This study was performed in accordance to the Declaration of Helsinki, and the protocol was approved by the local research ethics committee.

Genotyping

Genomic DNA was extracted by using 200 μL EDTA anticoagulated blood for isolation with a QIAamp blood mini kit (Qiagen, Leusden, the Netherlands) according to the manufacturers' instructions. Real-time polymerase chain reaction fluorescence resonance energy transfer assays were employed to determine the genotype for the CYP3A5 A6986G polymorphism.

The statistical analyses were performed by SPSS 20.0 (SPSS, Inc., Chicago, Ill, United States). Categorical data were expressed as percentages, and continuous data were expressed as mean ± standard deviation or median (range). Categorical data were compared with χ^2 or Fisher's exact tests; continuous data were compared with Student *t* test or Kruskal-Wallis test. All tests were 2-tailed, and differences for *P* values of .05 or less were considered significant.

Table 1. Basic Demographic and Clinical Parameters Between CYP3A5 Expressers and Nonexpressers

	CYP3A5 Expressers (n = 41)	CYP3A5 Nonexpressers (n = 45)	*P* Value
Age at transplant (years)	41.0 ± 9.0	43.2 ± 10.6	.31
Male, n (%)	21 (51.2)	26 (57.8)	.54
Causes of ESKD, n (%)			.99
Glomerulonephritis	23 (56.1)	25 (55.6)	
Diabetes	3 (7.3)	4 (8.9)	
Hypertension	4 (9.8)	4 (8.9)	
Unknown/others	11 (26.8)	12 (26.6)	
Deceased/Living transplant, n (%)	40 (97.6)/1 (2.4)	42 (93.3)/3 (6.7)	.62
Serum albumin (g/L)	43.0 ± 2.8	42.7 ± 3.5	.66
Serum alanine aminotransferase (IU/L)	19.5 ± 9.1	23.0 ± 17.1	.25
Hematocrit level (L/L)	0.38 ± 0.06	0.38 ± 0.06	.54
Daily tacrolimus dose (mg/kg) at 1 year	0.09 ± 0.03	0.05 ± 0.20	<.01
$dnAUC_{0\text{-}12}$ (ng×h/mL per mg) at 1 year	19.2 ± 7.2	40.4 ± 27.0	<.01

Abbrivations: $dnAUC_{0\text{-}12}$, dose-normalized 12-hour area under the curve; ESKD, end-stage kidney disease.

Table 2. Intrapatient Variability in Tacrolimus Abbreviated AUC_{0-12} Between CYP3A5 Expressers and Nonexpressers

Coefficient of Variation (%)	CYP3A5 Expressers (n = 41)	CYP3A5 Nonexpressers (n = 45)
Mean	16.7 ± 5.7	18.2 ± 7.5
Median	17.5	17.6
Range	7.4–28.4	5.0–38.6

Abbrivation: $dnAUC_{0-12}$, dose-normalized 12-hour area under the curve.

RESULTS

A total of 86 patients were included in our study. Among them, 83 patients (96.5%) had 5 paired blood samples (FK C_2 and C_4 levels) for calculation of the IPV of FK abbreviated AUC_{0-12}. The frequency distribution of CYP3A5*1/*1, CYP3A5*1/*3, and CYP3A5*3/*3 genotypes were 10.5% (n = 9), 37.2% (n = 32), and 52.3% (n = 45), respectively. Thus 41 patients (47.7%) were classified as CYP3A5 expressers while 45 were nonexpressers. There were no significant differences in the baseline characteristics between CYP3A5 expressers and nonexpressers (Table 1). The CYP3A5 expressers required 1.8 times higher FK dose compared with the nonexpressers.

The mean CV of FK $dnAUC_{0-12}$ for our whole cohort was 17.5 ± 6.7% (median 17.5%; range 5.0–38.6%). There was no significant difference in the mean CV between CYP3A5 expressers (16.7 ± 5.7%) and nonexpressers (18.2 ± 7.5%) (P = .31) (Table 2). Half of the expressers (n = 20) exhibited high CV of FK $dnAUC_{0-12}$ while half exhibited low CV. The same was evident in the nonexpresser group (23 high CV and 22 low CV). There was no significant difference using Fisher's exact test (P = 1). Finally, there was also no significant difference in the IPV of FK $dnAUC_{0-12}$ between patients with the CYP3A5*1/*1 versus CYP3A5*1/*3 versus CYP3A5*3/*3 (P = .47).

DISCUSSION

Instead of using C_0, our center is the first to use calculated abbreviated AUC_{0-12} to investigate the impact of CYP3A5 genetic polymorphism on IPV of FK pharmacokinetics. In accordance with some other studies, there was no significant association between the CYP3A5 genotype and IPV of FK concentrations [7,8]. On the other hand, Chung et al reported a higher proportion of CYP3A5 nonexpressers among those with a higher IPV of FK clearance. They hypothesized that in patients without a functional CYP3A5 enzyme, FK metabolism depends exclusively on the activity of CYP3A4, which is more sensitive to induction and inhibition [6]. However, the subjects involved in this study were only a small group of healthy volunteers who participated in a pharmacokinetic bioequivalence study involving 2 different FK formulations. The IPV shown in the study might be related to the difference of the 2 FK preparations. Furthermore, Stifft et al showed that it was the CYP3A5 expresser group who demonstrated a significantly higher IPV compared to the CYP3A5 nonexpresser group under a twice-daily FK regimen (CV AUC_{0-24} 12.6% in CYP3A5*3/*3 group vs 18.2% in CYP3A5*1/*3 group, P = .05). A major drawback was that they could only include a limited number of patients with the CYP3A5*1 allele, and none of them were homozygous for this allele [13]. On the other hand, although the size of our cohort was relatively small, all patients in our cohort were Chinese, and the percentage of CYP3A5*1 carrier was much higher when compared to the studies primarily on Caucasian patients.

Previous studies reported that there can be a wide range of FK IPV, with some individuals having an IPV of <5% while others have an IPV >50%. On average, FK IPV ranges between 15% and 30% [11]. In our cohort, the mean and median FK IPV was only 17.5%. On the other hand, the IPV of FK C_0 was 39.9 ± 19.8% in the African Americans, and this high IPV was found to be significantly associated with an increased risk of acute rejection in these patients. The authors postulated that the higher prevalence of CYP3A5*1 allele and medication nonadherence in the African Americans might explain this high FK IPV [4]. However, as shown in our study and other literature, the impact of CYP3A5 gene polymorphism on FK IPV is still not fully elucidated. It is more likely that nonadherence is the dominant cause of FK IPV. In addition, the absence of an association between FK IPV and the CYP3A5 genotype is consistent with the findings that the CYP3A5 genotype had no impact on acute rejection and long-term graft outcome for KTRs [14–17]. In fact, IPV could be considered as an objective measure of medication adherence and another way to monitor the effect of interventions designed to improve adherence [5].

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