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Original Article

Prediction of tacrolimus metabolism and dosage requirements based on CYP3A4 phenotype and *CYP3A5*3* genotype in Chinese renal transplant recipients

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Aim: To examine how the endogenous CYP3A4 phenotype and *CYP3A5*3* genotype of Chinese renal transplant recipients influenced the dose-corrected trough concentration (C_0/D) and weight-corrected daily dose (D/W) of tacrolimus.
Methods: A total of 101 medically stable kidney transplant recipients were enrolled, and their blood and urine samples were gathered. The endogenous CYP3A4 phenotype was assessed by the ratio of 6β-hydroxycortisol and 6β-hydroxycortisone to cortisol and cortisone in urine. *CYP3A5*3* genotype was determined using PCR-RELP.
Results: In overall renal transplant recipients, a multiple regression analysis including the endogenous CYP3A4 phenotype, *CYP3A5*3* genotype and post-operative period accounted for 60.1% of the variability in C_0/D ratio; a regression equation consisting of the endogenous CYP3A4 phenotype, post-operative period, body mass index, *CYP3A5*3* genotype, gender, total bilirubin and age explained 61.0% of the variability in D/W ratio. In *CYP3A5*3/*3* subjects, a combination of the endogenous CYP3A4 phenotype, post-operative period and age was responsible for 65.3% of the variability in C_0/D ratio; a predictive equation including the endogenous CYP3A4 phenotype, post-operative period, body mass index, gender and age explained 61.2% of the variability in the D/W ratio. Base on desired target range of tacrolimus trough concentrations, individual daily dosage regimen was calculated, and all the observed daily doses were within the predicted range.
Conclusion: This study provides the equations to predict tacrolimus metabolism and dosage requirements based on the endogenous CYP3A4 phenotype, *CYP3A5*3* genotype and other non-genetic variables.

Keywords: tacrolimus; CYP3A phenotype; *CYP3A5*3* genotype; Chinese renal transplant recipients

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Introduction

Tacrolimus (Tac) is a potent immunosuppressive agent that has been widely used to improve the outcome of organ transplantation. The need for frequent and specific monitoring of drug concentrations remains essential because the therapeutic dosing and pharmacokinetics of Tac show great variability among recipients[1]. Tac is known to be extensively metabolized by cytochrome P450 (CYP) 3A4 and CYP3A5. Intestinal P-glycoprotein (P-gp) is a product of the multidrug resistant 1 (*MDR1*) gene in humans and limits the bioavailability of Tac via active efflux[2, 3]. Importantly, most single nucleotide polymorphisms (SNPs) for CYP3A4 are unable to fully explain the inter-individual variability in CYP3A enzymatic activity[4]. In contrast, the *CYP3A5*3* SNP (A6896G) in exon 3 is strongly associated with CYP3A5 expression[5], and the effect of the *CYP3A5*3* allele on the pharmacokinetics of oral Tac has been confirmed by consistently positive results[1, 6]. Other reports have indicated that the most common SNPs for MDR1, including the synonymous SNPs C1236T in exon 12, C3435T in exon 26 and the nonsynonymous SNP G2677T/A in exon 21[7], play

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a minor role in the metabolism of Tac[1]. Hence, *CYP3A5*3* polymorphisms may be the only useful SNP for Tac dosage adjustment[6]. However, such genetic information is unlikely to be responsible for the residual inter-individual variability in the pharmacokinetics of Tac among CYP3A5 expressers or CYP3A5 non-expressers. Therefore, accurately formulating an individual's dosing regimen remains limited[6]. As a complementary or alternative strategy, determination of a patient's CYP3A4 phenotype may help to overcome the limitations of genetic assays and optimize Tac dosage regimens.

Currently, the ratio of 6β-hydroxycortisol to free cortisol (6β-OHF/F) in urine is thought to be a useful marker of both the induction and the inhibition of hepatic CYP3A4 activity[8]. The interconversion of cortisol (F) to cortisone (E) and 6β-hydroxycortisol (6β-OHF) to 6β-hydroxycortisone (6β-OHE) by 11β-hydroxysteroid dehydrogenase (11β-HSD) plays an important role in cortisol metabolism; thus, using cortisol as a CYP3A4 phenotyping probe may be confounded due to the regulation of cortisol feedback[9, 10]. Our previous experiments confirmed that the combined ratio of 6β-hydroxycortisol and 6β-hydroxycortisone to cortisol and cortisone in urinary MR reflects CYP3A4 catalytic ability and not the total activity of CYP3A4 and CYP3A5 isoforms (unpublished data). Another previous study completed by our laboratory showed that the MR was significantly related with the dose-corrected trough concentrations (C_0/D) of Tac in renal transplant recipients (r= -0.824, P<0.05)[11].

The major aim of our current study was to investigate the relationship between the endogenous CYP3A4 phenotype (assessed by urinary MR) and Tac metabolism and dosage requirements in Chinese renal transplant recipients. We also investigated the influence of *CYP3A5*3* genotype and other potential determinants of Tac disposition.

Materials and methods

Ethics statement

This study was performed in accordance with the declaration of Helsinki and its amendments. The experimental protocol was approved by the Ethical Committee of the School of Pharmaceutical Sciences at Central South University. The study is a part of registered clinical trial in ClinicalTrials.gov and the identifier is NCT01699360 (http://clinicaltrials.gov/show/NCT01699360). Written informed consents were obtained from all subjects before commencing the study.

Materials

The 6β-OHF, 6β-OHE, F, E and dexamethasone were purchased from Sigma-Aldrich (St Louis, MO, USA). They were of at least 98% purity. Acetonitrile, methanol and formic acid were of LC grade and purchased from Tedia (Tedia Company Inc, Fairfield, CT, USA). All other chemicals were of AR grade and obtained from commercial sources (Sinopharm Chemical Reagent Co Ltd, Shanghai, China).

Human subject study

The study was comprised of 101 medically stable kidney transplant recipients between 1 and 2 years after transplantation. A total of 231 specimens were collected at the 3rd Affiliated Hospital of Xiangya Medical Institute at Central South University. Patients who met the following criteria were included: receiving an immunosuppressive regimen containing Tac (tacrolimus capsules, Prograf®, Astellas Ireland Co Ltd, Ireland), mycophenolate mofetil (CellCept®, Roche, Shanghai, China) and prednisolone; oral administration of Tac at twice the daily dose at least 5 d prior to the study; undergone no more than one renal transplant; normal liver and renal function; and older than 18 years of age. The following exclusion criteria were used for this study: an acute rejection episode or infection; multiple organ transplantation; use of any other medications known to induce or inhibit the CYP3A enzyme or interact with immunosuppressive agents, with the exception of amlodipine; or any abnormal findings on physical examinations or laboratory tests.

At the time of investigation, the dose of Tac ranged between 1.0 and 8.0 mg/day. The doses of mycophenolate mofetil and prednisolone were 0.36–1.5 mg/day and 2.0–30.0 mg/day, respectively. On the morning of the study, urine specimens were collected between 8:00 AM and 10:00 AM. Blood samples were obtained before Tac administration. Blood samples were immediately sent to the clinical laboratory for Tac analysis and *CYP3A5*3* genotyping, and urine samples were stored at -20 °C for subsequent analysis of 6β-OHF, 6β-OHE, F and E. All subjects underwent routine laboratory tests, including hematology, blood chemistries and urinalysis. The demographics of each subject and their co-medications were also recorded (Table 1). Each patient was instructed to eat his or her usual breakfast, excluding caffeine- and grapefruit-containing foods, and to take any other morning medication except Tac.

Table 1. The data of renal transplant recipients. Mean±SD.

Parameter	Value
Age (year)	37.4±9.9
Gender (male/female)	77/24
Body weight (kg)	60.2±10.5
Height (cm)	165.7±6.4
Body mass index (kg/m^2)	21.8±3.2
Postoperative periods (h)	11 887.6±2032.6
Glutamic-pyruvic transaminase (U/L)	25.2±16.8
Glutamic oxalacetic transaminase (U/L)	21.2±12.3
Total bilirubin (μmol/L)	13.2±4.3
Blood urea nitrogen (mmol/L)	6.1±2.0
Serum creatinine (μmol/L)	97.3±47.6
Serum uric acid (μmol/L)	337.4±70.1
Comedication of amlodiping (with/without)	50/51

*CYP3A5*3* genotyping

Genomic DNA was extracted from peripheral lymphocytes according to the instructions provided with the Genomic DNA

Purification Kit (Promega, Madison, WI, USA). Identification of *CYP3A5*3* genotypes were performed using the previously reported polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay[12]. The forward primer was 5′-CATGACTTAGTAGACAGATGAC-3′ and the reverse primer was 5′-GGTCCAAACAGGGAAGAAATA-3′. The amplified DNA fragment was 293 bp and was digested with *Ssp* I (Fermentas, Vilnius, Lithuania) at 37 °C for 4 h. The genotype for each individual was further validated by a sequencing assay (data not shown).

Analytical methods

6β-OHF, 6β-OHE, F and E in urine

The 6β-OHF, 6β-OHE, F and E in urine were measured using the HPLC-UV method developed in our laboratory[11]. The lower limit of quantification was 5 μg/L for all of the compounds in the urine. The accuracy was determined at three concentrations and ranged between 98.2% and 115.5%.

Trough concentrations of tacrolimus in whole blood

Trough concentrations of Tac in whole blood were assayed by the Microparticle Enzyme Immunoassay (MEIA, Abbott, Princeton, NJ, USA) using blood samples taken on the morning of the study. The assay was performed according to the instructions supplied with the Tacrolimus II monoclonal antibody kit (Abbott, Princeton, NJ, USA).

Data and statistical analysis

The normality distribution of parameters was assessed using the Kolmogorov-Smirnov test. The C_0/D ratio and weight-corrected daily dosage (D/W) of Tac between *CYP3A5*3* genotypes was compared using a one-way ANOVA followed by *post-hoc* Bonferroni corrected *t*-tests. The relationship between the C_0/D and D/W ratios of Tac and urinary MR were analyzed by linear regression. Correlations of the C_0/D and D/W ratios of Tac with each factor (as shown in Table 1) were analyzed with an α=0.05. Factors that were not statistically significant were rejected. A stepwise multiple regression analysis with statistically significant factors was used to obtain the coefficient of determination (R^2). The models for the prediction of the C_0/D and D/W ratios of Tac were constructed based on the multiple regression equation. All statistical analysis was performed using SPSS 17.0 statistics software, and a *P* value <0.05 was considered significant.

Results

Effect of *CYP3A5*3* genotype on the C_0/D and D/W ratios of Tac

The Kolmogorov-Smirnov test illustrated that the logarithm of C_0/D and D/W ratios of Tac and the logarithm of urinary MR, postoperative periods, body mass index and total bilirubin were normally distributed. The values of all other continuous variables (as shown in Table 1) showed a normal distribution in Chinese renal transplant recipients.

The C_0/D ratios of Tac in the *CYP3A5*3/*3* group were significantly higher when compared with those in the *CYP3A5*1/*1* and *CYP3A5*1/*3* groups (Supplementary Table S1). Consistent with the relationship between CYP3A5 genotype and phenotype, the D/W ratios of Tac in *CYP3A5*3/*3* subjects were markedly lower when compared with *CYP3A5*1* carriers (*CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes) (Supplementary Table S1).

The relationship between the endogenous CYP3A4 phenotype and tacrolimus metabolism

As shown in Figure 1, the endogenous CYP3A4 phenotype (assessed by urinary MR) significantly correlated with the C_0/D and D/W ratios of Tac. Within *CYP3A5*3/*3* subjects, urinary MR was related to the C_0/D and D/W ratios; however, only weak correlations were observed in *CYP3A5*1* carriers (*CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes).

Multiple regression analysis for the prediction of tacrolimus metabolism and dosage requirements

Final regression models were established to predict the C_0/D and D/W ratios of Tac (Table 2). The coefficients of determination (R^2) were 0.601 and 0.610 for the C_0/D and D/W ratios, respectively. Within the *CYP3A5*3/*3* genotype, the values of R^2 were 0.653 and 0.612 for the C_0/D and D/W ratios, respectively (Table 3). According to equations (1) and (3), the daily dosage regimen was determined:

Table 2. The regression analysis for the prediction of the C_0/D and D/W ratios of Tac in overall renal transpant recipients (*n*=231).

No	Predicted value	Regression equation	R^2	*P*
Equation (1)	C_0/D ratio ng·mL^{-1}/(mg·d^{-1})	Log_{10} C_0/D=0.331−0.338*Log_{10} MR−0.083*CYP3A5+0.046*Log_{10} POT	0.601	<0.001
Equation (2)	D/W ratio (mg·d^{-1}·kg^{-1})	Log_{10} D/W=0.303+0.230*Log_{10} MR−0.133*Log_{10} POT−0.750* Log_{10} BMI +0.081* CYP3A5+0.066*gender−0.133*Log_{10}TBIL−0.002*age	0.610	<0.001

C_0/D, dose-corrected trough concentration of Tac; D/W, weight-corrected stable dose of Tac; Log_{10} MR, the logarithm of the ratio of 6β-hydroxycortisol and 6β-hydroxycortisone to cortisol and cortisone in urine; *CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes were set at the value of "1"; *CYP3A5*3/*3* genotype was set at the value of "0"; Log_{10} POT, the logarithm of post-operative period; Log_{10} BMI, the logarithm of body mass index; gender, male was defined as the value of "0"; female was defined as the value of "1"; Log_{10} TBIL, the logarithm of total bilirubin; R^2, coefficient of determination; *P*, probability.

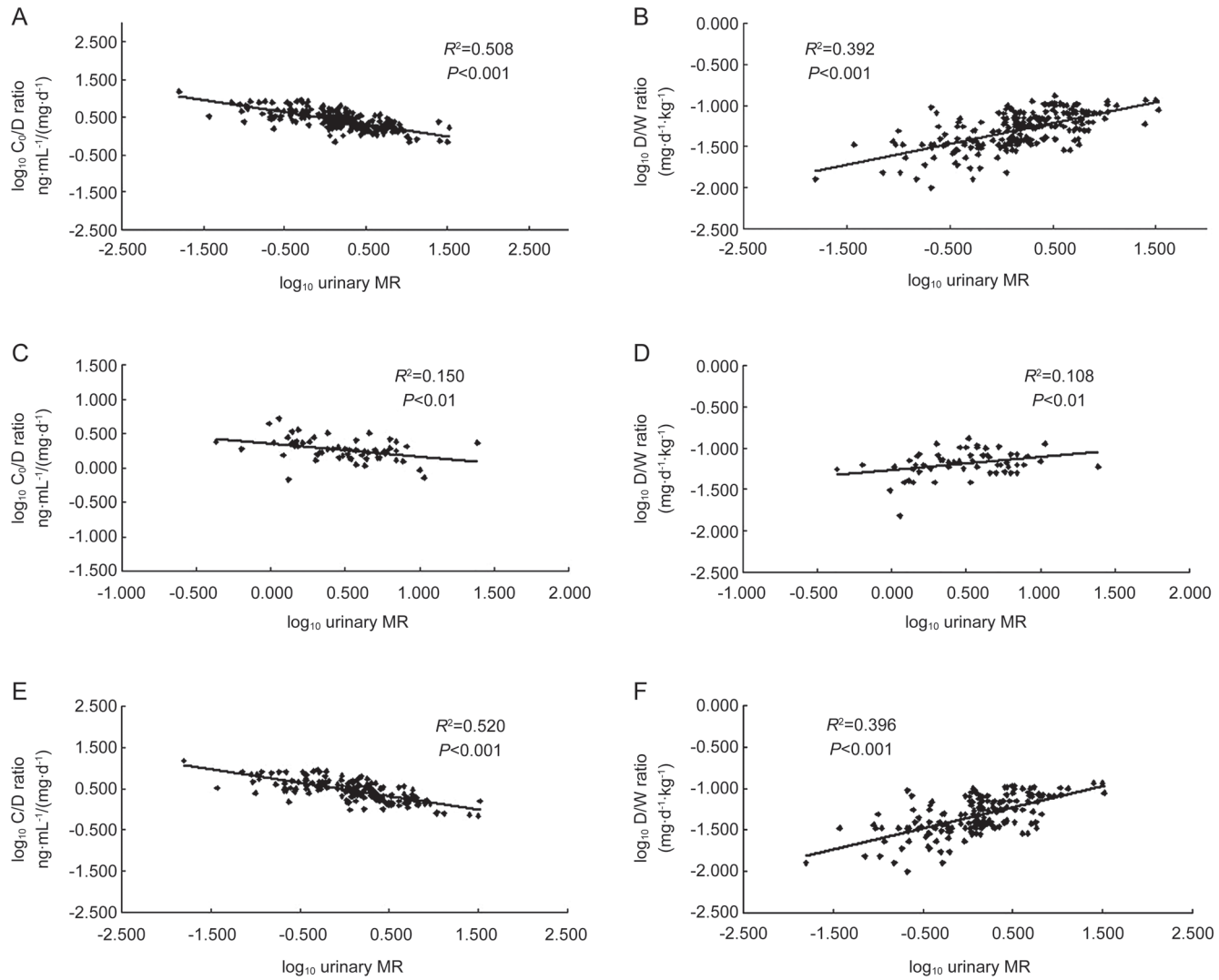

Figure 1. Linear regression relationships between urinary MR and the C_0/D and D/W ratios of tacrolimus across the different *CYP3A5*3* genotypes. (A) with the C_0/D ratio in overall subjects (*n*=231); (B) with the D/W ratio in overall subjects (*n*=231); (C) with the C_0/D ratio in *CYP3A5*1* carriers (*n*=58); (D) with the D/W ratio in *CYP3A5*1* carriers (*n*=58); (E) with the C_0/D ratio in *CYP3A5*3/*3* genotype (*n*=173); (F) with the D/W ratio in *CYP3A5*3/*3* genotype (*n*=173).

Daily dosage regimen for all subjects=C_0/Power [10, (0.331–0.338*Log_{10}MR–0.083*CYP3A5+0.046*Log_{10}POT)]

Equation (5)

C_0, trough concentration of Tac; Log_{10}MR, the logarithm of the ratio of 6β-hydroxycortisol and 6β-hydroxycortisone to cortisol and cortisone in urine; *CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes were set at "1"; the *CYP3A5*3/*3* genotype was set at "0"; Log_{10}POT, the logarithm of post-operative period.

Daily dosage regimen for *CYP3A5*3/*3* subjects=C_0 /Power [10, (0.132–0.357*Log_{10}MR+0.074*Log_{10}POT+0.003*age)]

Equation (6)

C_0, trough concentration of Tac; Log_{10}MR, the logarithm of the ratio of 6β-hydroxycortisol and 6β-hydroxycortisone to cortisol and cortisone in urine; Log_{10}POT, the logarithm of post-operative period.

Based on the desired target range of Tac C_0 levels, an individual's daily dosage regimen was directly calculated by equation (5) and (6). The results illustrate that all of the observed doses were within the predicted dosage range (Table 4).

Discussion

Therapeutic drug monitoring (TDM) and *CYP3A5*3* genotyping are common tools for the adjustment of an individual's Tac dosage in the clinic. However, TDM can be labor intensive because this method requires the frequent monitoring of drug concentrations in blood. Notably, TDM cannot predict an individual's dosage; thus, many patients can have adverse reactions before drug monitoring begins[1]. *CYP3A5*3* genotyping can help to optimize the Tac dose regimen between CYP3A5 expressers and CYP3A5 non-expressers. Our results confirm previous reports showing that *CYP3A5*3* polymor-

Table 3. The regression analysis for the prediction of the C_0/D and D/W ratios of Tac in renal transpant recipients with *CYP3A5*3/*3* genotype (*n*=173).

No	Predicted value	Regression equation	R^2	*P*
Equation (3)	C_0/D ratio ng·mL^{-1}/(mg·d^{-1})	Log_{10} C_0/D=0.132–0.357*Log_{10} MR+0.074*Log_{10} POT+0.003*age	0.653	<0.001
Equation (4)	D/W ratio (mg·d^{-1}·kg^{-1})	Log_{10} D/W=0.333+0.251*Log_{10} MR–0.151* Log_{10} POT–0.820*Log_{10} BMI +0.061*gender–0.002*age	0.612	<0.001

C_0/D, dose-corrected trough concentration of Tac; D/W, weight-corrected stable dose of Tac; Log_{10} MR, the logarithm of the ratio of 6β-hydroxycortisol and 6β-hydroxycortisone to cortisol and cortisone in urine; Log_{10} POT, the logarithm of post-operative period; Log_{10} BMI, the logarithm of body mass index; gender, male was defined as the value of "0"; female was defined as the value of "1"; R^2, coefficient of determination; *P*, probability.

Table 4. The desired target range of Tac concentration, the predicted daily dosage range and the observed daily dose of Tac in overall (*n*=231) and *CYP3A5*3/*3* subjects (*n*=173). Mean±SD.

Subjects	Desired target range of concentration (ng·mL^{-1})[a]	Predicted daily dosage range (mg·d^{-1}) Lower bound	Upper bound	Observed daily dose (mg·d^{-1})
Overall	3–7	1.0±0.4	3.7±1.5	3.4±1.5
*CYP3A5*3/*3* genotype	3–7	0.9±0.4	3.2±1.6	3.1±1.5

[a]The desired target range of concentration was set according to the clinical practice in the 3rd Affiliated Hospital of Xiangya Medical Institute, Central South University;

phisms play an important role in Tac metabolism[1, 6]. CYP3A5 non-expressers (*CYP3A5*3/*3*) displayed higher C_0/D ratios and lower D/W ratios when compared with CYP3A5 expressers (*CYP3A5*1/*1* and *CYP3A5*1/*3*) (Supplementary Table S1). Within CYP3A5 expressers or CYP3A5 non-expressers, there was a residual inter-individual variability in the dosage requirements and pharmacokinetics of Tac. Therefore, we hypothesized that *in vivo* CYP3A4 phenotyping was an independent predictor of Tac disposition.

A previous study suggested that 56%–59% of the variability in Tac dose requirements and clearance could be explained by *in vivo* CYP3A4 activity assessed by midazolam clearance and CYP3A5 genotype[6]. Administration of midazolam is not convenient because of its pharmacological effects as a central nervous system depressant; thus, taking advantage of endogenous substances, such as markers of the CYP3A4 phenotype, can avoid unnecessary administration of probe drugs to humans. In our study, a non-invasive CYP3A4 phenotype was used by determining the metabolic ratio of endogenous 6β-hydroxymetabolites to cortisol and cortisone. No serial blood sampling for the CYP3A4 phenotype was needed. The findings showed that this endogenous CYP3A4 probe explained 50.8% and 39.2% of variability in the C_0/D and D/W ratios, respectively, of all subjects (Figure 1). The multiple regression analysis, including CYP3A4 phenotype assessed by urinary MR, *CYP3A5*3* genotype and post-operative period, accounted for 60.1% of the variability in the C_0/D ratio (Table 2). Combination of the *in vivo* CYP3A4 phenotype, post-operative period, body mass index, *CYP3A5*3* genotype, gender, total bilirubin and age explained 61.0% of variability in the D/W ratio (Table 2). These results are similar to a previous report on midazolam clearance and *CYP3A5*3* genotype[6].

Within CYP3A5 non-expressers (*CYP3A5*3/*3*), urinary MR was responsible for 52.0% and 39.6% of the variability in the C_0/D and D/W ratios of Tac, respectively; however, only a 15.0% and 10.8% contribution was found among CYP3A5 expressers (*CYP3A5*1/*1* and *CYP3A5*1/*3*) (Figure 1). Furthermore, 65.3% and 61.2% of the variance in the C_0/D and D/W ratios, respectively, was explained by the multiple regression equations presented in Table 3. Our previous study suggested that urinary MR reflects CYP3A4 activity (unpublished data), and the metabolism of Tac depends on a total of CYP3A4 and CYP3A5 activities[13]. Among *CYP3A5*3/*3* subjects, we assumed that all compounds were mainly catalyzed by the CYP3A4 isoform, and the absence of the CYP3A5 enzyme may contribute to the marked correlation between urinary MR and Tac disposition.

The recommended blood concentration of Tac is 3–7 ng/mL at least 3 months after transplantation. The dosage requirements should be carefully controlled in order to achieve the desired concentration of Tac. Using equations (5) and (6), the desired daily dosage was predicted, and all of the observed doses were within the predicted range (Table 4). Our findings suggest that equations (5) and (6) can provide additional valuable information for the use of Tac in the clinic.

In our study, all patients received an immunosuppressive regimen containing Tac, mycophenolate mofetil and prednisolone. Prednisolone can be converted to prednisone, which is a weak inducer of CYP3A4[14]. However, we found that a varying dosage of prednisolone had no significant effect on Tac disposition and CYP3A4 phenotype (data not shown). Furthermore, calcium-channel blockers were more commonly

used after transplantation. Amlodipine does not interact with the CYP3A enzyme at therapeutic doses[14] and was included in the study population. Our results suggest that co-medication with amlodipine did not affect the C_0/D and D/W ratios of Tac in Chinese renal transplant recipients (data not shown).

Tac is metabolized by the CYP3A enzyme, but it can also be influenced by the patient's physical condition after transplantation. Thus, only patients with a similar postoperative period were selected. In our study, patients at a stable stage (between 1–2 years after transplantation) were enrolled. As a result, only stable doses were investigated, and further studies should focus on the prediction of initial dosage requirements in the early post-operative period.

In summary, we developed equations that describe the association between the endogenous CYP3A4 phenotype and Tac metabolism and dosage requirements. These equations take into account the *CYP3A5*3* genotype and other potential variables. Within *CYP3A5*3/*3* genotypes, 65.3% and 61.2% of the inter-individual variability in Tac metabolism and dosage requirements were explained by the endogenous CYP3A4 phenotype and other non-genetic variables. These findings provide a potential basis for estimating a Tac dosing regimen using a non-invasive CYP3A4 probe, rather that the administration of probe drugs or serial blood sampling. Furthermore, our predictive algorithm based on phenotype and genotype can be used prior to treatment.

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Author contribution

Xi LUO and Ze-neng CHENG designed the research; Xi LUO, Li-jun ZHU and Li-yun ZHENG performed the research; Li-jun ZHU and Ze-neng CHENG contributed analytic tools; Xi LUO, Ning-fang CAI and Li-yun ZHENG analyzed the data; and Xi LUO and Ze-neng CHENG wrote the paper.

Supplementary information

Supplementary Table S1 is available at Acta Pharmacologica Sinica's website.

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