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Original Article

Relationships of related genetic polymorphisms and individualized medication of tacrolimus in patients with renal transplantation

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Abstract: The aim of this study was to establish clinical and genetic factors-based individual administration model of tacrolimus for Chinese Han patients after renal transplantation (RT). The genetic polymorphisms of CYP3A4, CYP3A5 and MDR1 in 216 RT patients were detected by PCR-RFLP, the genetic and clinical factors and blood concentration/dose × body weight (C/D) values of tacrolimus were performed the single factor correlation analysis, and established the dose prediction algorithm of tacrolimus by stepwise multiple regression analysis. CYP3A5*3, hematocrit and albumin were correlated with the C/D values of tacrolimus, the best regression model could explain 28.3% reason of individual dose differences of tacrolimus, among which CYP3A5*3 polymorphism could explain 23.5%. The genetic factors played an important role in the dose differences of tacrolimus, the patients should be checked CYP3A5*3 genotype before administration of tacrolimus to predict the tacrolimus doses, thus helping to improve the safety and effectiveness of tacrolimus application.

Keywords: Tacrolimus, genetic polymorphism, CYP3A4, CYP3A5, MDR1, renal transplantation, individualized medication

Introduction

Tacrolimus was the most widely used calcineurin inhibitor after RT, but because of its narrow therapeutic window, big individual pharmacokinetic differences, its medication dose must be constantly adjusted through blood concentration monitoring to remain it within the therapeutic concentrations [1, 2]. In current clinical practice, it would take a few weeks to adjust to the maintenance dose of tacrolimus, during which period the RT patients might face higher risks of transplant rejection or renal toxicity, so it was very important to achieve a stable maintenance dose as soon as possible [3].

Study had shown that 20%-95% of individual differences in drug response and disposal were caused by genetic factors [4]. Tacrolimus was the substrate of drug transportation protein P-glycoprotein, CYP3A4 and CYP3A5 [5], the expression difference of CYP3A4, CYP3A5 and P-gp was one of the important reasons that led to the high pharmacokinetic difference of tacrolimus [6-8]. CYP3A5 genetic polymorphism was related with the pharmacokinetic difference of tacrolimus, especially the third intron 6986A > G (rs7767746), the mutation of this site could cause the variable splicing of pre-mRNA, resulting in an unstable protein, therefore the patients carrying this mutations would not express the CYP3A5 metabolic enzyme, only carrying at least one*1 allele, could active CYP3A5 be expressed [9]. A number of studies indicated that the patients with CYP3A5*1 genotype required higher dose of tacrolimus to achieve the goal blood concentration than those with CYP3A5*3/* 3 genotype [10-12]. So far, 39 SNP of CYP3A4 gene had been identified, among which CYP3A4*1B (392A > G; rs2740574), located in the promoter region, was related with tacrolimus metabolism and had been in-depth studied, this mutation might affect the activities of metabolic enzymes [13].

However, the occurrence frequency of CYP3A4*1B in China population was almost zero, so this mutation might not be the main cause that resulted in the individual pharmacokinetic differences of tacrolimus in Chinese population. In 2004, Japanese scientists found, through large-scale sequencing, CYP3A4*18B (rs2242480) located in 10th intron [14], this mutation could improve CYP3A4 activities [15]. P-gp could affect intestinal absorption, distribution, metabolism and excretion, and was the product encoded by multi-drug resistant MDR1 gene [16], the 26th exon 26 3435C > T (rs1045642), the 21st exon 2677G. T/A (rs2032585) and the 12th exon 1236C > T (rs1128503) were the polymorphisms mostly studied, and these mutations might lead to the pharmacokinetic differences of tacrolimus [17, 18].

Several recent studies used CYP3A5 genotype to guide tacrolimus dose in RT patients [19]. Thervet reported that the initial dose of tacrolimus towards the patients carrying one or more CYP3A5*1 alleles in the genotype guiding group was 0.3 mg/kg/d, while the initial dose towards those without CYP3A5*1 allele was 0.15 mg/kg/d; the initial dose of the non-genotype guiding group was 0.2 mg/kg/d. As for the patient ratios that reached the target concentration 3 days after administration, the genotype guiding group was greater than the non-genotype guiding group (43.2% vs 29.1; P = 0.03), and 75% patients could reach the target concentration faster with fewer dose adjustment times. Chen also conducted the similar study [20].

Furthermore, Pamala collected tacrolimus doses, plasma concentrations and clinical data within postoperative 6 months of 681 RT cases from multiple observation centers in USA and Canada [21], the researchers found that CYP3A5*1 genotypes, transplantation days, ages, steroid sparing center and calcium channel blocker (CCB) had significant impacts on tacrolimus CL/F, the final model was CL/F (l h-1) = 38.4 × [(0.86, if days 6-10) or (0.71, if days 11-180)] × [(1.69, if CYP3A5*1/* 3 genotype) or (2.00, if CYP3A5*1/* 1 genotype)] × (0.70, if receiving a transplant at a steroid sparing centre) ¥ ([age in years/50] -0.4) × (0.94, if CCB is present). Because of the ethnic diversities, combined with the genetic characteristics of Chinese Han population, the drug and non-drug genomic factors that would impact absorption and metabolism of tacrolimus in RT patients should be fully considered, the retrospective study design, multivariate analysis principles and methods, combined with genetic factors (CYP3A4*18B, CYP3A5*3, MDR1 C1236T, G2677T/A and C3435T) and non-genetic factors (age, sex, liver and kidney functions, albumin and hemoglobin, etc.), should be considered together to build the dose prediction algorithm of tacrolimus, aiming to develop the most appropriate regimen for this patient when firstly administrated, thus shortening the dose adjustment time of tacrolimus in RT patients, reducing the risks caused by multiple dose adjustments-induced concentration fluctuations, and achieving safe, effective, personalized and economic medication of tacrolimus.

Materials and methods

The patients that were firstly performed renal transplantation and regularly followed up in certain third-grade class A hospital of Fuzhou from October 2005 to April 2011 were selected, a total of 216 cases (all Han nationality) were selected, including 160 males and 56 females, aged 18 to 65 years old (39 ± 11 years); the body weights were 33 kg-81 kg, with the average as (58.2 ± 9.4) kg; the heights were 146 cm-192 cm, with the average as (166.2 ± 7.4) cm. The patients with preoperative liver functional abnormalities, combined with the administration of nephrotoxic drugs (such as amphotericin B and aminoglycosides, etc.), and administrated CYP3A enzyme inducers or inhibitors (e.g. rifampicin and macrolides, etc.) two weeks before treatment were excluded. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Nanjing Command PLA. Written informed consent was obtained from all participants.

Immunosuppressive regimen

The immunosuppressive regimen used the triple therapy of tacrolimus + mycophenolate mofetil + corticosteroids, meanwhile, all patients were administrated diltiazem with a fixed dose (30 mg, qd) to increase the plasma concentrations of tacrolimus. Tacrolimus: dose: 0.1 $mg \cdot kg^{-1} \cdot d^{-1}$-0.12 $mg \cdot kg^{-1} \cdot d^{-1}$, orally administrated 1 h before meals or 2 h after meals, the first dose was administrated in the post-RT

Table 1. PCR amplification primers and restrictive enzymes

Polymorphism	Primer	Restrictive enzyme
CYP3A4*18B	F_1: 5'-CAC CCT GAT GTC CAG CAG AAA CT-3'	Rsa I
	F_2: 5'-AAT AGA AAG CAG ATG AAC CAG AGC C-3'	
CYP3A5*3	F_1: 5'-CAT GAC TTA GTA GAC AGA TGA-3'	Ssp I
	F_2: 5'-GGT CCA AAC AGG GAA GAA ATA-3'	
MDR1C1236T	F_1: 5'-TAC CCA TCT CGA AAA GAA GTT AAG G-3'	Hae III
	F_2: 5'-GAA AGA TGT GAA TGT GAC TGC TGA T-3'	
MDR1G2677T	F_1: 5'-TGC AGG CTA TAG GTT CCA GG-3'	Ban I
	F_2: 5'-TTT AGT TTG ACT CAC CTT CCC G-3'	
MDR1G2677A	F_1: 5'-GCA GGA GTT GTT GAA ATG AAA ATGT-3'	Rsa I
	F_2: 5'-GGG GAG GAA GGA AGA ACA GTGT-3'	
MDR1C3435T	F_1: 5'-TGC TGG TCC TGA AGT TGA TCT GTG AAC-3'	Mbo I
	F_2: 5'-ACA TTA GGC AGT GAC TCG ATG AAG GCA-3'	

2nd evening, and adjusted according to the blood concentration monitoring results, the goal treatment concentration was (5 ng/mL-10 ng/mL). Mycophenolate mofetil: dose: 0.5 g•d^{-1}-2 g•d^{-1}, orally administrated from the post-RT 1st day. Hormone administration: on the post-RT 0-3rd day, 500 mg•d^{-1} methylprednisolone sodium succinate (methylprednisolone) was routinely administrated intravenously, and changed to oral administration of 20 mg•d^{-1} prednisone from the post-RT 4th day.

Extraction of whole blood DNA

2 mL-3 mL peripheral venous blood was collected from each patient and kept in EDTA-anticoagulation tube at 4°C, the whole blood was centrifuged at 3000 r•min^{-1} for 5 min, discarded the upper plasma, and extracted DNA with the modified potassium iodide method within a week. One UV spectrophotometry (protein and nucleic acid analyzer, Eppendorf, Germany) was used to measure the extracted DNA's OD260 and OD280, then calculated OD260/OD280, which was within 1.6-2.0 could indicate the qualified purity.

Genotype analysis

The methods of polymerase chain reaction (PCR) and restrictive fragmentation length polymorphism (RFLP) were performed to analyze the genotypes of CYP3A4*18B, CYP3A5*3, MDR1 C1236T, MDR1 G2677T/A and MDR1 C3435T. the PCR primers of all loci were designed and shown in **Table 1**, the PCR reaction system was 25 μL: 2.5 μL 10 × PCR reaction buffer, 2 μL deoxyribonucleic acid triphosphate (dNTP) (2.5 mmol•L^{-1}), 0.5 μL forward and reverse primer (20 mmol•L^{-1}), respectively, 0.125 μL Taq DNA polymerase (5 U•μL^{-1}), 2.0 μL genome DNA, then added double distilled water to make the total reaction volume as 25 μL. PCR reaction conditions were as follows: 94°C denaturation for 5 min, 94°C denaturation for 30 s, 54°C-63°C annealing for 30 s, 72°C extension for 30 s, 35 cycles, and finally 72°C extension for 5 min. The products were digested with restrictive endonuclease at 37°C for 4 h-16 h. the digestion products were performed electrophoresis with EB-stained 3% agarose gel at constant 100 V for 30 min, the electrophoresis results were then observed by gel imager (WD-9413B gel imaging analysis system, Beijing 61 equipment manufacturer).

Clinical data

Checked the patients' medical records, and recorded the following information: demographic data (age, gender, height and weight); medication situations (tacrolimus, mycophenolate mofetil, prednisone, diltiazem and other concomitant medications); the whole blood trough concentration of tacrolimus and biochemical indicators (creatinine, urea, alanine aminotransferase, total bilirubin, albumin, hemoglobin and hematocrit) were detected on the post-RT 5th day; maintenance dose of tacrolimus (referring to the dose that could maintain the trough concentration within therapeutic window for 2 consecutive measurements, and the interval time was at least 14 days or more), trough concentration and biochemical indicators at this time.

Determination of whole blood trough concentration

3 ml of venous blood was sampled from the patients with renal transplantation at post-medication 24 h or when in the stable status

Table 2. Mutation frequencies of CYP3A4, CYP3A5 and MDR1 gene polymorphism in 216 RT patients

Polymorphism	Genotype	Cases	Genotype frequency	Allele	Allele frequency
CYP3A4*18B (rs 2242480)	*1/*1	101	46.7%	*1	69.2%
	*1/*18B	97	44.9%	*18B	30.8%
	*18B/*18B	18	8.4%		
CYP3A5*3 (rs 776746)	*1/*1	17	7.9%	*1	25.8%
	*1/*3	77	35.7%	*3	74.2%
	*3/*3	122	56.4%		
MDR1C1236T (rs 1128503)	C/C	25	11.4%	C	34.4%
	C/T	99	45.8%	T	65.6%
	T/T	92	42.7%		
MDR1G2677T/A (rs 2032582)	G/G	54	25.1%	G	50.9%
	G/A	26	11.9%	T	38.8%
	G/T	86	39.6%	A	10.3%
	A/A	3	1.3%		
	T/T	34	15.9%		
	A/T	13	6.2%		
MDR1C3435T (rs 1045642)	C/C	78	36.1%	C	61.7%
	C/T	110	51.1%	T	38.3%
	T/T	28	12.8%		

Table 3. Correlations of indicators with tacrolimus C/D value

Independent variable	Correlation Coefficient (r)	Coefficient of Determination (R^2)	*P*
Sex	0.033	0.0011	0.623
Age	0.118	0.0139	0.081
Height	0.004	0.0000	0.958
Mycophenolate mofetil	0.057	0.0032	0.398
Prednisone	0.078	0.0061	0.252
Urea	0.097	0.0094	0.151
Creatinine	0.094	0.0088	0.165
Albumin	-0.171	0.0292	0.011
Transaminase	0.097	0.0094	0.152
Total bilirubin	0.030	0.0009	0.658
Hemoglobin	0.169	0.0286	0.012
Hematocrit	0.176	0.0310	0.009
CYP3A4*18B	-0.354	0.1253	0.000
CYP3A5*3	0.574	0.3295	0.000
MDR1 C1236T	0.010	0.0001	0. 879
MDR1G2677T/A	0.018	0.0003	0.788
MDR1 C3435T	0.021	0.0004	0.761

before medication in the morning, and ELISA was performed to detect the whole blood valley concentration. In order for the convenient comparison, the blood concentrations of tacrolimus were performed the dose calibration, namely the plasma concentration/dose × body weight (C/D) was set as the evaluation index.

Statistical analysis

All data were analyzed with SPSS17.0 statistical software, Sperman's correlation was used to analyze the correlations of each factor with C/D values, respectively, and the factors with statistical significance were set as independent variables, C/D value was set as the dependent variable for the multiple linear regression analysis and establishing the multiple regression equation, with $P < 0.05$ considered as the statistically significant difference.

Results

Genotyping

The DNA genotyping results of the 216 RT patients by PCR-RFLP were shown in **Table 2**. The mutation frequencies of CYP3A4*18B, CYP3A5*3, MDR1 C1236T, MDR1 G2677T/A and MDR1 C3435T allele in RT patients were 30.8%, 74.2%, 65.6%, 38.8%, 10.3% and 38.3%, respectively, the Hardy-Weinberg test analysis revealed $P > 0.05$, the frequency of each gene acquired genetic equilibrium, and the study subjects had the group representation.

Table 4. Corresponding correlations among CYP3A4*18B and CYP3A5*3 genotype with dummy variables

Dummy variable	Genotype 1	Genotype 2	Genotype 3
CYP3A5*3	*1/*1	*1/*3	*3/*3
X_1	0	0	1
X_2	0	1	0
CYP3A4*18B	*1/*1	*1/*18B	*18B/*18B
X_3	0	0	1
X_4	0	1	0

Table 5. Multiple linear regression analysis of indicators and tacrolimus C/D

Variable	Coefficient	Standard error	t	*P*
(Constant)	86.350	38.272	2.256	0.025
X1	72.053	9.026	7.983	< 0.001
HCT	2.658	0. 781	3.406	0.001
ALB	-2.097	1.009	-2.079	0.039

Ingle-factor correlation analysis of each indicators and tacrolimus C/D value

Referring the methods of univariate and multivariate analysis, the three demographic indexes (gender, age and height), 9 clinical parameters (urea, creatinine, albumin, alanine aminotransferase, total bilirubin, hemoglobin, hematocrit combining with mycophenolate mofetil and hormone) and five genetic factors (CYP3A4*18B, CYP3A5*3, MDR1 C1236T, G2677T/A and C3435T) were defined as the independent variables, and the C/D value was defined as the dependent variable (Y) for the univariate correlation analysis, the results were shown in **Table 3**. Five indicators were correlated with tacrolimus C/D, among which albumin and CYP3A4*18B were negatively correlated with tacrolimus C/D, while hemoglobin, hematocrit and CYP3A5*3 were positively correlated with tacrolimus C/D. The other 12 indicators showed no significant correlation with tacrolimus C/D ($P > 0.05$).

Multiple regression analysis of each index with tacrolimus C/D

Albumin, hemoglobin, hematocrit, CYP3A4*18B, CYP3A5*3 and tacrolimus C/D were performed stepwise regression analysis, the arrangements of such dummy variables as CYP3A4*18B and CYP3A5*3 were shown in **Table 4**. In the best regression model, the factors that had statistical significance towards tacrolimus C/D were CYP3A5*3, hematocrit and albumin, the results were shown in **Table 5**, CYP3A5*3 was the first factor introduced into the equation, and it could explain 23.5% of individual differences; followed by hematocrit, which could explain 3.3%, and the third was albumin, and it could explain 1.5%. The multiple regression equation, obtained from the best regression model, was namely the tacrolimus C/D prediction algorithm (**Table 6**), and could explain 28.3% of individual differences. The formula was as follows.

$$Y = 86.350 + 72.053 \times X_1 + 2.658 \times HCT - 2.097 \times ALB$$

Among the equation, Y (blood concentration/dose × body weight (C/D) of tacrolimus) was the dependent variable, so the tacrolimus dose prediction model was:

$$Dose/weight\ (D) = C/(86.350 + 72.053 \times X_1 + 2.658 \times HCT - 2.097 \times ALB)$$

X1 represented the CYP3A5*3 polymorphism, CYP3A5-expression patients (type *1/*1 or type*1/*3) × 0, CYP3A5 non-expression type (*3/*3) × 1. C was the clinical therapeutic drug monitoring target (5 ng•mL^{-1}-10 ng•mL^{-1}).

Prediction effectiveness of individualized medication model of tacrolimus

According to the follow-up records, the 216 RT patients obtained the corresponding indexes for maintaining the doses, which were then substituted into the tacrolimus dose prediction model: dose/body weight (D) = C/(86.350 + 72.053 × X_1 + 2.658 × HCT-2.097 × ALB), if C (clinical therapeutic drug monitoring target) was within 5 ng•mL^{-1}-10 ng•mL^{-1}. The prediction value of D was within 0.05 ± 0.02 mg•kg^{-1}-0.09 ± 0.04 mg•kg^{-1}, the actual D was 0.08 ± 0.03 mg•kg^{-1}, and in the dose range predicted by tacrolimus dose prediction model; if the patient's actual tacrolimus blood concentration was introduced into the prediction model, the mean predicted D value would be 0.06 ± 0.03 mg•kg^{-1}, showing significant difference with the actual D value ($P < 0.01$), the predicted dose/weight value was lower than the actual one by

Table 6. Multiple regression model of individualized medication of tacrolimus

Model	Variable	Regression equation	*P*	R^2
1	X_1	$Y = 81.142 + 73.552 \times X_1$	< 0.001	0.235
2	X_1, HCT	$Y = 20.076 + 73.640 \times X_1 + 2.317 \times HCT$	0.003	0.268
3	X_1, HCT, ALB	$Y = 86.350 + 72.053 \times X_1 + 2.658 \times HCT\text{-}2.097 \times ALB$	0.039	0.283

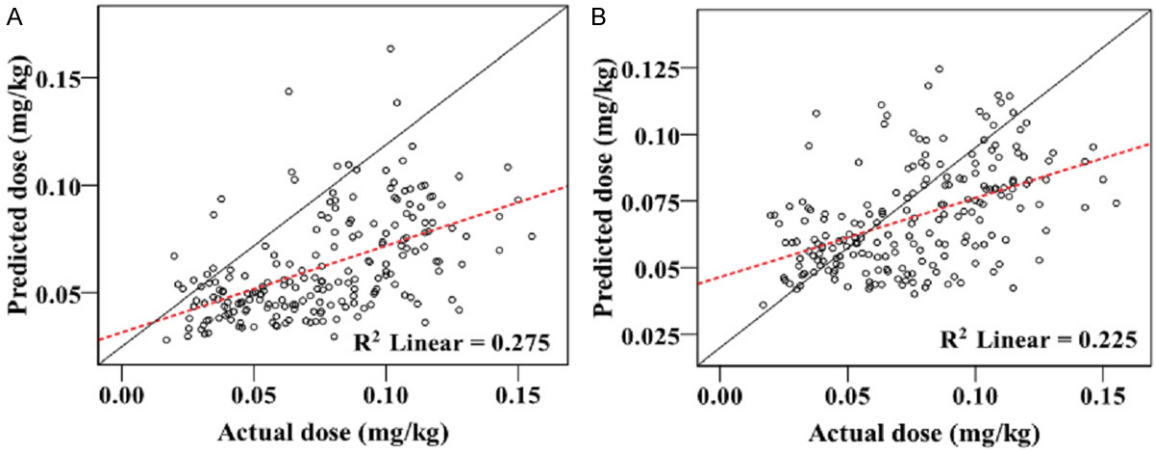

Figure 1. Correlations of predicted and actual maintenance doses.

0.01 ± 0.03 mg•kg^{-1}. **Figure 1A** was the scatterplot of predicted and actual D values, R^2 = 0.275, the tacrolimus dose prediction model could explain 27.5% of tacrolimus dose/weight difference.

Discussion

Tacrolimus was the first-line immunosuppressant in preventing transplantation rejection, the current clinical practice normally administrated the same body-weight dose, then made adjustments to this initial dose based on the TDM monitoring results, but because of its narrow therapeutic window and big pharmacokinetic difference, it would require the patients to adjust the doses within bigger range and longer time, and the incidences of acute rejection and adverse reactions were higher, thus it would seriously affect the treatment efficacies of RT and the patients' life qualities.

As for the factors that caused the individual differences of Tacrolimus, this study showed that three days after administration, the main factors that would impact bacrolimus blood concentration were CYP4*18B, CYP3A5*3, hemoglobin, hematocrit and albumin, these factors were performed stepwise regression analysis with tacrolimus C/D values, respectively, and the best regression model was introduced CYP3A5*3, hematocrit and albumin to establish the individualized dosing model of tacrolimus: dose/body weight (D) = C/(86.350 + 72.053 × X_1 + 2.658 × HCT-2.097 × ALB), this model could explain 28.3% of tacrolimus dose/weight difference, among which the CYP3A5*3 polymorphism could explain 23.5%, playing an important role towards the individualized differences of tacrolimus blood concentrations. In order to verify the effectiveness of this prediction model, this study recorded 216 RT patients and performed follow-up survey to obtain the corresponding indexes of maintenance doses, which were then substituted into the model for the validation, the results showed that the predicted tacrolimus D was lower than the actual value, and the difference was statistically significant (R^2 = 0.275), if C (clinical therapeutic drug monitoring target) inside the model was within 5 ng•mL^{-1}-10 ng•mL^{-1}, the predicted D value inside the model would be 0.05 ± 0.02 mg•kg^{-1}-0.09 ± 0.04 mg•kg^{-1}.

In the early post-RT period, the RT patients would have many kinds of medications, including antibiotics, liver-protective drugs, gastric mucosa-protective drugs and blood-activation

drugs and so on, most of these drugs needed to be metabolized by liver, which would variously impact the metabolism of tacrolimus, some drugs that needed the oral administration might interfere with the absorption of tacrolimus inside the gastrointestinal tract, thus competing with tacrolimus; secondly, the uremic patients had poor physical conditions, after suffering from the shocks of major surgeries and large amounts of liquid input, the patients' internal environment would exhibit dramatic changes, and these would all impact the absorption and metabolism of tacrolimus, so the fitting degree obtained in this research was lower (28.3%). Due to complexities of the patient with renal transplantation himself as well as the related medications, there was rare report about individual administration model of tacrolimus in China or abroad, the present study attempted to fit them together, the resulted model was only suitable for the triple immunosuppressive regimen of tacrolimus, corticosteroids and mycophenolate mofetil, while as for the patients with diltiazem, there were more limitations, and the fit degree was low, so it needed future prospective trials to further adjust and optimize this program in order to work out the best administration model, aiming to be able to reduce dose adjustment amplitude of tacrolimus in the patients with renal transplantation, shorten dose adjustment time, reduce adverse reactions and rejection rates, and improve quality of life and survival rates in the patients with renal transplantation, thus really realizing the individualized treatment of tacrolimus.

Disclosure of conflict of interest

None.

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