

Full-length article

Influence of CYP3A5 genetic polymorphism on cyclosporine A metabolism and elimination in Chinese renal transplant recipients

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Key words

cyclosporine; genetic polymorphism; CYP3A5; metabolic ratio; renal transplant patients

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Abstract

Aim: To investigate whether the *CYP3A5**3 polymorphism would affect cyclosporine A (CsA) metabolism in Chinese renal transplant patients. **Methods:** The *CYP3A5**3 genotype was determined in Chinese renal transplant recipients using polymerase chain reaction and amplification of specific alleles (PCR-ASA). The concentrations of CsA and metabolites were separately measured by fluorescence polarization immunoassay and dose-adjusted trough concentrations and metabolic ratio (MR) values were calculated. **Results:** The trough concentrations adjusted with the dose was significantly higher in the wild allele carriers compared to both the homozygous (*3*3) and heterozygous variants (*1*3). However, no significant difference was found for the dose-adjusted metabolite concentrations. The MR values for the 3 genotype groups were as follows: 0.92±0.62 for *CYP3A5**3/*3 (*n*=14), 0.99±0.51 for *CYP3A5**1/*3 (*n*=15), and 1.45±0.62 for *CYP3A5**1/*1 (*n*=9), respectively. *Post hoc* comparisons showed that only the MR values between the *CYP3A5**3/*3 group and the *CYP3A5**1/*1 group were significantly different. **Conclusion:** The *CYP3A5**3 polymorphism exerted little effect on cyclosporine metabolism. The MR may be a more accurate indicator for therapeutic drug monitoring, considering its integrated information on body exposure of both parent drugs and metabolites.

Introduction

Cyclosporine was the most frequently used immunosuppressant in organ transplant patients. However, it has a narrow therapeutic index which demands therapeutic drug monitoring followed by dose adjustment. Furthermore, the pharmacokinetic characteristics of cyclosporine were subject to high inter-patient variations.

The interindividual differences in the expression of drug metabolizing enzymes were the major factor contributing to pharmacokinetic variations^[1]. The cytochrome P450 3A (CYP3A) subfamilies are the most abundant and important drug-metabolizing enzymes in humans and participate in the metabolism of 45%–60% of currently used drugs and a variety of other compounds^[2]. A wide interindividual variability in CYP3A expression and function has been identified, and polymorphic expression of CYP3A5 was one of the major

contributors^[3,4]. Single nucleotide polymorphisms (SNP) in *CYP3A5**3 and *CYP3A5**6 that cause alternative splicing and protein truncation resulted in the absence of CYP3A5 from the tissues of some individuals^[4,5]. CYP3A5 was believed to be the most important genetic contributor to interindividual and interracial differences in CYP3A-dependent drug clearance and responsive to many medicines^[5]. Since immunosuppressant cyclosporine A (CsA) was a known substrate for CYP3A5^[6], we sought to investigate whether the *CYP3A5**3 polymorphism, which accounts for more than 70% of gene frequency in Chinese people, is associated with CsA metabolism and elimination in Chinese renal transplant patients.

Although the influence of CYP3A5 polymorphism on the pharmacokinetics of cyclosporine had been studied and reported by several research groups, the results and conclusions have been controversial^[7–10]. Furthermore, most of

the reported studies determined only the dose-adjusted trough concentrations, and no one had an insight into metabolite formation. In fact, it has been recognized that the CsA concentration, or dose requirement, was poorly associated with the clinical outcomes^[11]. Therefore, we determined both CsA and its metabolite concentrations for evaluating the influence of the CYP3A5 polymorphism on cyclosporine's elimination and metabolism. The metabolic ratio (MR) of CsA vs metabolites was used as an indicator showing the integrated effect of the CYP3A5 polymorphism on CsA metabolism and elimination.

Materials and methods

Human subjects The study protocol was approved by the ethical committee of the General Hospital of Nanjing Military Area. One-hundred-and-forty-seven Chinese (Han nationality) renal transplant recipients receiving CsA were genotyped for CYP3A5*3 polymorphism. All subjects (at least 6 months after first transplants) received the same immunosuppressive treatment (cyclosporine and dexamethasone), and took no drugs known to interfere with CsA disposition. The selected subjects had stable kidney graft function and laboratory values, without significant or new abnormal findings in physical examination or medical history. The CsA dose (Sandimmun Neoral, Novartis, Beijing, China;

microemulsion formulation) was required to be constant during the last 5 d before the onset of the study. Blood samples from all the patients prior to the next dose were collected for determining CsA trough concentrations. The patients (38 subjects) who had good clinical compliance and whose age ranged from 30 to 40 were selected for the total metabolite determination. The demographic data of the subjects are summarized in Table 1.

DNA isolation and genotyping The whole blood samples were obtained by venous puncture from the subjects and DNA was extracted from each sample (250 μL) using a DNA isolation kit (provided by Anhui-Gen Biotechnology Co Ltd, Hefei, China). The CYP3A5*3 genotype was then determined by polymerase chain reaction and amplification of specific alleles (PCR-ASA)^[7]. The primers of CYP3A5*3 were designed according to its sequence in GENBANK (AC005020); the PCR-ASA conditions used in the genotyping are listed in Table 2 and Table 3, respectively. PCR-ASA was performed using a Perkin-Elmer GeneAmp PCR System 2400 (Wellesley, MA, USA). Representative detection of the CYP3A5*3 polymorphism by PCR-ASA is depicted in Figure 1.

Determination of CsA and metabolite concentrations The whole blood concentrations of CsA and its total metabolites were measured by fluorescence polarization immunoassay^[12] run on a TDX analyzer (Abbott, Abbott Park, Illinois, USA)

Table 1. Demographic data of the subjects.

CYP3A5*3 genotypes	*3/*3 group (n=14)	*3/*1 group (n=15)	*1/*1 group (n=9)	Range
Age	40±11	39±9	40±9	25~50
Sex (m/f)	58/16	53/7	8/5	
Weight (kg)	60±6	57±7	59±6	48~74
Cyclosporine A dose (mg/d)	286±85	288±114	228±95	100~450
ALT/AST ^a	Normal	Normal	Normal	

^a Transaminase

Table 2. Primers used to identify CYP3A5 variants.

Allele (location)	Primers	Sequence	DNA size of PCR
CYP3A5*3	AF	5'-CCTGCCTTCAATTTTTCACT-3'	1443 bp
	BR	5'-GCAATGTAGGAAGGAGGGCT-3'	
(A22893 → G)	CF-W:	5'-AGAGCTCTTTGTCTTTCAA-3'	518 bp
	CF-M:	5'-AGAGCTCTTTGTCTTTTCAG-3'	
	DR:	5'-CAAGATTGCAAGATGTTACC-3'	

Table 3. PCR-ASA conditions used in genotyping.

Step and PCR product	PCR-ASA conditions	Cycles
Step 1 (1443 bp)	94 °C-2 min	35 cycles
	94 °C-1 min	
	57 °C-0.5 min	
	72 °C-1.5 min	
	72 °C-10 min	
Step 2 (518 bp)	94 °C-2 min	12 cycles
	94 °C-1 min	
	65 °C-1 min	
	72 °C-1 min	
	72 °C-10 min	

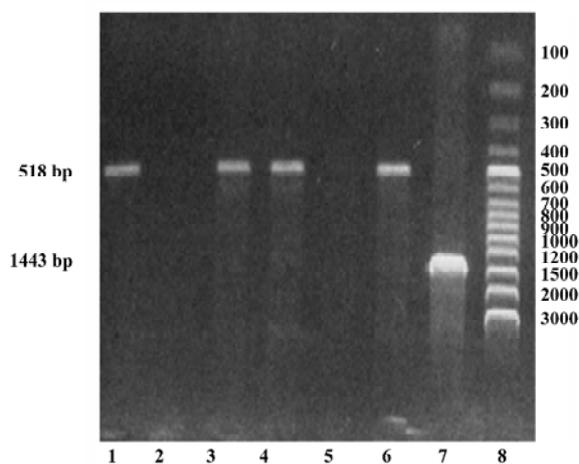


Figure 1. Detection of *CYP3A5*3* polymorphism by PCR-ASA. Lanes 1 and 2 are from the samples of the homozygous *G/G* genotype. Lanes 3 and 4 are from the samples of the heterozygous *G/A* genotype. Lanes 5 and 6 are from samples of the homozygous *A/A* genotype. Lane 7 is from the sample of the first step PCR (1443 bp). Lane 8 is a marker.

following the manufacturer’s instructions. The whole blood reagent packs for CsA and metabolites were purchased from Abbott. The assay intraday and interday variations were determined to be within 15% and accuracy was close to 100%.

The 38 selected subjects were divided into the *CYP3A5*3/*3* group (*n*=14), *CYP3A5*I/*3* group (*n*=15) and *CYP3A5*I/*I* group (*n*=9) according to genotyping. Both concentrations of cyclosporine and its metabolites were dose adjusted. The MR value was calculated by using the following equation^[8]:

$$MR = C_{CsA} \text{ (mg}\cdot\text{L}^{-1}) / C_{Met} \text{ (mg}\cdot\text{L}^{-1})$$

Statistical analysis In order to differentiate the potential influence of *CYP3A5* genotype on the metabolism and the elimination of cyclosporine, we separately compared the dose-adjusted trough concentrations of the parent drug and its metabolites and the MR. Samples were grouped according to their *CYP3A5*3* genotypes, and the statistical differences were determined using one-way ANOVA followed by *post hoc* multiple comparisons based on the least squares means (SAS 6.12).

Results

Genotyping Of the 147 patients in the study, 74 were found to be *CYP3A5*3* homozygous carriers, 60 were *CYP3A5*I*3* carriers and only 13 patients were the wild-type **I*I* carriers. The *CYP3A5*3* gene frequency was determined to be 71% in this study in accordance with previously published reports. From the selected 38 subjects for further metabolite determination, 14, 15 and 9 were determined to be *CYP3A5*3/*3*, *CYP3A5*I/*3* and *CYP3A5*I/*I*, respectively.

Trough concentrations of CsA, metabolites and MR The trough concentration adjusted with the dose was significantly higher in the wild allele carriers as compared to both the homozygous (**3*3*) and heterozygous variants (**I*3*). However, no significant difference was found when the dose-adjusted metabolite concentrations were compared between the different groups. Based on the trough concentrations of CsA and its metabolites, the MR values were calculated. The MR values for the 3 genotype groups were as follows: 0.92 ± 0.62 for *CYP3A5*3/*3* (*n*=14), 0.99 ± 0.51 for *CYP3A5*I/*3* (*n*=15), and 1.45 ± 0.62 for *CYP3A5*I/*I* (*n*=9), respectively. The result of the ANOVA analysis indicated weak statistical evidence of difference among the 3 groups (*P*=0.0882). Further *post hoc* comparisons with least squares means showed that only the MR values between the *CYP3A5*3/*3* group and the *CYP3A5*I/*I* group were significantly different (*P*=0.0328; Table 4, 5).

Discussion

The *CYP3A5*3* variants were known to cause a significant lower hepatic and jejunal *CYP3A5* mRNA expression as compared to the *CYP3A5*I*I* genotype^[13]. However, research on the influence of *CYP3A5* gene polymorphism on *CYP3A* enzyme activity have been greatly inconsistent^[7-10]. Some studies conducted *in vivo* supported that the *CYP3A5*3* genotype resulted in slower CsA oral clearance as compared to the wild-type allele. In contrast, Yates *et al* reported that CsA oral clearance was significantly 37.87%

Table 4. Trough concentrations of CsA in different CYP3A5 genotype carriers.

Groups	Genotype	n	Age	Dose (mg·kg ⁻¹ ·d ⁻¹)	C _{CsA} (ng/mL)	C _{trough} /Dose (ng/mL per mg·kg ⁻¹ ·d ⁻¹)
1	*I*I	M8 F5	40±9	3.94±2.05	381.12±381.56	86.98±50.04
2	*I*3	M53 F7	39±9	3.73±1.63	250.39±301.71	56.73±42.46
3	*3*3	M58 F16	40±11	2.93±1.24	175.50±154.54	47.28±24.26
	<i>p</i> ₁₋₃				0.0059	0.0011
	<i>p</i> ₂₋₃				0.0728	0.3303
	<i>p</i> ₁₋₂				0.0871	0.0071

Table 5. Trough concentrations of cyclosporine and its metabolites, and MR value in different CYP3A5 genotype carriers.

Groups	Genotype	Dose (mg·kg ⁻¹ ·d ⁻¹)	C _{trough} /Dose (ng/mL per mg·kg ⁻¹ ·d ⁻¹)	C _{Met} /Dose (ng/mL per mg·kg ⁻¹ ·d ⁻¹)	MR value
1	*I*I	3.88±1.59	122.05±58.60	88.55±30.33	1.45±0.62
2	*I*3	5.16±2.23	93.95±70.82	99.97±63.12	0.99±0.51
3	*3*3	4.87±1.58	65.44±32.98	82.95±39.08	0.92±0.62
	<i>p</i> ₁₋₃		0.0073	0.7247	0.0328
	<i>p</i> ₂₋₃		0.3998	0.7865	0.0840
	<i>p</i> ₁₋₂		0.0432	0.9102	0.5943

lower in the *CYP3A5**I*I genotype compared to the *CYP3A5**3*3 genotype (30.1±2.8 vs 41.5±3.1L/h, *P*=0.027)^[14]. The possible cause for such a contradictory conclusion may be that the different parameters such as dose-adjusted trough concentrations, dose adjusted area under the curve (AUC) and dose requirements were compared in the different research. The contradictory results obtained from different parameters highlighted the importance of selecting more accurate parameters for accessing the influence of genotype on pharmacokinetics.

In the present study, we determined both CsA and its metabolite concentrations. The statistical comparison of the dose-adjusted metabolite concentrations showed no significant difference among the 3 CYP3A5 genotypes. However, significant differences were found when trough concentrations of CsA were compared among the 3 groups or between the wild-type and *CYP3A5**3 variant carriers. These results indicate that the *CYP3A5**3 variants exert no significant influence on the metabolism of cyclosporine. However, it seems to be controversial that the trough concentrations of CsA were significant lower in the *CYP3A5**3 carriers compared to the wild-type *CYP3A5* carriers. The possible influence of gender on the CsA pharmacokinetics and metabolism were statistically compared within the 3 CYP3A5 genotype groups

and no significant difference was observed, which was inconsistent with the previous report^[15].

It was argued that lower plasma concentrations of parent drugs might be caused by a more rapid metabolism (including first pass metabolism which occurs in the intestines or liver) or elimination itself. Cyclosporine was a well known substrate of P-glycoprotein of which the higher expression was the main cause for the lower bioavailability or faster oral clearance of cyclosporine. MDR1 (multidrug resistance gene), the gene encoding P-glycoprotein was also subject to high frequency of polymorphism. It was reported that CsA oral clearance was significantly higher in subjects who carried the 3435T allele as compared to the wild-type individuals (40.0±2.2 vs 26.4±3.1 L/h, *P*= 0.007), and the MDR1 C3435T genotype accounted for approximately 43% of the interindividual variability in CsA oral disposition. It was interesting to find that the *CYP3A5**3 allele carriers were more likely to carry the MDR1 3435T allele than the 3435C allele (*P*=0.038); *CYP3A5**3 G/G genotype individuals were 3435T allele carriers^[14]. Combining the previous reports and our present study, we can conclude that the CYP3A5 polymorphism exerts little effect on cyclosporine metabolism; however, mechanisms underlying the *CYP3A5**3-associated rapid clearance remains unknown.

Furthermore, interindividual differences in the systemic profile of cyclosporine metabolites were found to be responsible to the divergent adverse drug response to a therapeutic systemic CsA exposure level^[16]. Therefore, determining only the trough concentrations or the AUC of CsA was not enough for therapeutic monitoring. We highlighted in the present study that MR values, taking both the parent drugs and metabolites into account, should be a more accurate indicator for therapeutic monitoring and for the evaluation of gene polymorphism influence on drug exposure. The statistical data of our study indicates that only the MR values between *CYP3A5**3*3 and *CYP3A5**1*1 group are significantly different ($P=0.0328$), with MR values in the *3*3 carriers 36.03% lower than that of *1*1 carriers.

In conclusion, the *CYP3A5**3 polymorphism exerted little effect on cyclosporine metabolism. The MR should be a more accurate parameter for determining the body exposure of both parent drugs and potential active metabolites.

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