Association Between Tacrolimus Concentration and Genetic Polymorphisms of CYP3A5 and ABCB1 During the Early Stage After Liver Transplant in an Iranian Population

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Abstract

Objectives: **Tacrolimus is widely used as an immunosuppressive drug in liver transplant recipients with a narrow therapeutic range and variable individualized pharmacokinetics. Tacrolimus is a substrate of cytochrome P-450 3A enzyme and the drug transporter, P-glycoprotein.**

Materials and Methods: **We determined the genotypic frequencies of cytochrome P-4503A5 (rs776746), and ABCB1 (rs1045642), single nucleotide polymorphisms in a population of 100 Iranian liver transplant patients, and investigated the influence of the above-mentioned single nucleotide polymorphisms on tacrolimus concentrations. At 7 and 30 days after transplant, tacrolimus dosages (mg/kg/d), trough blood levels (T0), and dose-adjusted concentrations (concentration/dosage ratio) were determined. Polymerase chain reaction, followed by restriction fragment length polymorphism analysis, was used for genotyping cytochrome P-4503A5*3 [6986A>G] as well as ABCB1 [3435C>T].**

Results: **Ninety-five percent of the population showed a cytochrome P-4503A5*3/*3 genotype. ABCB13435TT genotype was observed in 33 cases (33%); whereas 51 cases (51%) carried 3435CT, and 16 cases (16%) carried 3435CC. With regard to the ABCB1 and cytochrome P-4503A5, they showed no influence on tacrolimus dosing requirements at 1 week or 1 month after transplant. No association of any genetic variant with the acute rejection rate was found.**

Conclusions: **Finally, as the liver donor genotype influences tacrolimus pharmacokinetics with regard to expression of cytochrome P-4503A5, far more than the genotype of the recipient; therefore, it should be considered before recommending any personal immunosuppressive treatment based on pharmacogenetics.**

Key words: *Pharmacogenetics, Immunosuppressive drugs, Organ, Transplant, Ethnicity*

Introduction

Tacrolimus, a calcineurin inhibitor, is widely used as an immunosuppressive drug in liver transplant recipients. The drug has a narrow therapeutic index with variable individualized pharmacokinetics.[1] Therefore, monitoring of trough blood concentrations is important to limit its toxicity with optimal immunosuppressive effect. Daily dosages are adjusted according to whole-blood trough concentrations. Many factors like organ function and drug interactions may influence the drug effects. Genetic factors are another factor responsible for interindividual variations of drug disposition.[2, 3] Tacrolimus is a substrate for P-glycoprotein (P-gp), the product of the *ABCB1* gene (the multidrug resistance, MDR). P-glycoprotein is expressed in many organs and systems like the intestine, the liver, the kidney, and the blood-brain barrier. This protein acts as a transmembrane efflux pump to export xenobiotics from inside the cells, decrease absorption

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from gut lumen, and increase biliary and urinary excretion. The high expression in the enterocyte is responsible for decreased intestinal absorption of drugs such as tacrolimus.[2, 3] ABCB1 is polymorphically expressed, with at least 50 SNPs identified to date.[4-6]

Although certain SNPs are associated with variability in P-gp transport capacity, no known ABCB1 SNP results in a total loss of P-gp expression or function. The most-commonly studied ABCB1 SNPs include a C to T transition at position 3435 within exon 26 (3435C>T), (rs1045642), a C to T transition at position 1236 within exon 12 (1236C>T), (rs1128503), and a G to T or A transition at position 2677 within exon 21 (2677G>T/A), (rs2032582) of the ABCB1 gene.[7]

ABCB1 2677G>T/A is believed to be a nonsynonymous SNP, which results in amino acid alterations in the encoding protein. ABCB1 3435C>T and 1236C>T are synonymous SNPs, whereby the nucleotide substitution does not change in the amino acid sequence of the encoded protein. Any influence of the synonymous SNPs (ABCB1 3435C>T and 1236C>T) may be due to linkage disequilibrium with a nonsynonymous SNP (ABCB1 2677G>T/A). On the other hand, the silent ABCB1 3435C>T SNP may decrease ABCB1 mRNA stability and level.[8]

Previous studies have evaluated the association of tacrolimus absorption and ABCB1 expression.[9, 10]

The cytochrome P-450 *(CYP)3A* isoenzymes have been identified as major enzymes responsible for the oxidative metabolism of this drug. *CYP3A5* contributes to 50% of the CYP3A subfamily activity.[11-13] It is also polymorphically expressed, with at least 11 reported SNPs until now.[14]

*CYP3A5**1 (6986A) is the most-important allele responsible for genetic polymorphism. The A to G substitution at 6986 nucleotide in CYP3A5 gene intron 3 (rs776746), CYP3A5*3 allele, results in the absence of functional *CYP3A5* activity in liver tissue. The variant allele was named CYP3A5*3 and the wild-type was assigned CYP3A5*1. Heterozygous or homozygous carriers of the CYP3A5*1 wild-type allele produce high levels of CYP3A5 mRNA and protein (CYP3A5 expressors). Homozygous carriers of the CYP3A5*3 variant allele produce very low or undetectable levels of CYP3A5 protein (CYP3A5 nonexpressors).[13, 14]

The literature shows a strong relation between the CYP3A5 6986A>G SNP, and tacrolimus pharmacokinetics has been reported in kidney and liver transplant recipients.[15, 16] The CYP3A5*1/*3 polymorphism is thought to contribute to the interindividual variation of tacrolimus pharmacokinetics.[2, 3] Inheritance of the *CYP3A5*1* allele is strongly associated with enhanced in vitro CYP3A5-dependent metabolism in the liver.[9]

Therefore, these SNPs may explain the large interindividual variations in the pharmacokinetics of tacrolimus. In this study, we investigated the effect of these 2 SNPs on tacrolimus dosages and trough levels in Iranian liver transplant recipients.

Materials and Methods

One hundred patients who underwent liver transplant from living and deceased donors between December 2005 and April 2006, who were treated with tacrolimus-based immunosuppressive regimens, were included in this study (Table 1).

All patients were Iranian and were transplanted at the Transplantation Center of Nemazi Hospital affiliated with the Shiraz University of Medical Sciences. The Ethics Committee of Shiraz University of Medical Sciences approved the protocol, and

Table 1. Demographic and clinical characteristics of transplant recipients (n=100).

Parameter	Value
Age (y)	35.1 ± 17.55, (1 to 60)
Sex (male/female) n (%)	64 (64) / 36 (36)
Body weight (kg)	49.17 ± 18.14, (8 to 70)
Donor age (y)	35.28 ± 10.81, (19 to 49)
Donor sex (male/female) n (%)	70 (70) / 30 (30)
Donor type	
Deceased	80 (80)
Living	20 (20)
Cause of cirrhosis n (%)	
Hepatitis B virus	17 (17)
Primary sclerosing cholangitis	15 (15)
Wilson disease	9 (9)
Autoimmune hepatitis	8 (8)
Progressive familial intrahepatic cholestasis	6 (6)
Biliary atresia	6 (6)
Hepatitis C virus	4 (4)
Tyrosinemia	3 (3)
Familial hypercholesterolemia	2 (2)
Crigler-Najar syndrome	2 (2)
Cryptogenic	28 (28)
Immunosuppression therapy n (%)	
Tacrolimus + cyclosporine + steroid†	82 (82)
Tacrolimus + mycophenolate + steroid†	18 (18)
Tacrolimus level, T0 (ng/mL)	
7 days	6.47 ± 4.99
1 month	9.13 ± 4.14

†The patient received steroid as pulse therapy for a short time.

written informed consent was obtained from all subjects. All protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration. For all patients, the initial dosage of tacrolimus was 0.15 mg/kg/d, administered orally, twice daily, cyclosporine 3 to 5 mg/kg/d or mycophenolate mofetil (20 mg/kg/d), and a steroid. Blood tacrolimus levels were measured 12 hours after the previous dose (T_0) by the Behring Syva EMIT method (enzyme multiplied immunoassay technique) in a whole blood sample. During the first month, the daily dosage was adapted to the target blood trough concentration 7 to 10 ng/mL. Transplant recipients weight (kg) and daily dosage (mg/d) of tacrolimus at 7 days and 1 month after transplant were recorded; the dosage per weight (mg/kg/d) and dosage-adjusted concentration (concentration/dosage ratio) also were calculated. Any pathologic or clinical episode of early acute rejection was recorded.[17]

Patients taking medication that affected cyclosporine/tacrolimus blood levels, such as diltiazem, verapamil, phenytoin, erythromycin, or clarithromycin, were excluded.

Genotyping assays

For genotype determination, genomic DNA was extracted from peripheral blood samples using a commercial extraction kit (DNP DNA Extraction Kit, Cinagene Company, Tehran, Iran).

To genotype the *A6986G* polymorphism in the *CYP3A5* gene, the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was used according to Tsuchiya and associates method with modification.[10] The PCR was carried out in 25 μL solution consisting of 40 to 50 ng DNA, 1.5 mM dNTP, 10 pmol each primer, and 1 U Taq polymerase. The primers used were as follows:

forward, 5'- ATGGAGAGTGGCATAGGAGATA-3';

reverse, 5'-TGTGGTCCAAACAGGGAAGAAA TA- 3'. Polymerase chain reaction amplification conditions were 10 minutes of initial denaturation at 94°C, followed by 40 cycles of melting at 94°C for 30 seconds, annealing at 65°C for 45 seconds, and elongation at 72°C for 30 seconds. The PCR products were digested with SspI (USA New England BioLabs Ltd., Pickering, Ontario, Canada) and visualized on 2.5% agarose gel. When the A allele (*CYP3A5* *1 allele) was present, the 130 bp PCR fragment was divided into 107 bp and 23 bp fragments.

A PCR-RFLP also was used for detecting C3435T SNP. The primers and PCR amplification protocol were designed according to Ameyaw and associates with some modification.[11] The primers used were as follows:

MDR1 F (5'-TGC TGG TCC TGA AGT TGA TCT GTG AAC-3') and

MDR1 R (5'-ACA TTA GGC AGT GAC TCG ATG AAG GCA-3').

The PCR amplification conditions were 10 minutes of initial denaturation at 94°C, followed by 35 cycles of melting at 94°C for 30 seconds and annealing at 68°C for 30 seconds. This was followed by digestion of a 248 bp PCR product with restriction enzyme Mbo I (Fermentase, Germany) for 2 hours at 37°C. Digested products were separated on a 2.0% agarose gel. Mbo I digestion of wild-type DNA yields fragments of 172 bp, 60 bp, and 16 bp. The C3435T mutation destroys 1 restriction site and Mbo I digestion yields a 238 bp and 16 bp fragments

Statistical Analyses

Data are expressed as means ± SD. Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 14.0, IBM Corporation, Armonk, New York, USA). Differences between the groups were assessed using an analysis of variance (ANOVA), and results were considered significant when the values for *P* were less than .05. The observed genotype frequencies were compared with expected genotype frequencies according to the Hardy-Weinberg equilibrium with Arlequin software (version 3.1).

Results

Frequency of ABCB1 and CYP3A5 variants in liver transplant recipients

Of the 100 liver transplant recipients, the *ABCB1*3435TT genotype was observed in 33 cases (33%); whereas 51 cases (51%) carried 3435CT, and 16 cases (16%) carried 3435CC. The allelic frequencies of T and C were 58.5% and 41.5%.

Respecting *CYP3A5*, 95 of the recipients (95%) exhibited a *3/*3 genotype, whereas 5 cases (5%) carried *1/*3. Nobody inherited a *1/*1 genotype. The allelic frequencies of *CYP3A5* *1 and *3 were 2.5% and 97.5%. There was no statistical difference of recipients' sex among the *ABCB1* or *CYP3A5* genotypes. Allele and genotype frequencies for the 2

genes were derived from the Hardy-Weinberg equilibrium.

Effect of ABCB1 and CYP3A5 SNP on tacrolimus dosage requirement

During the first month after liver transplant, the required tacrolimus dosages ranged from 0.152 to 0.193 mg/kg/d, with interindividual variations in tacrolimus pharmacokinetics.

After 1 week of adjusted dosages, the mean T_0 level reached the target therapeutic range, and no significant differences were observed among the 3 groups of ABCB1 gene (P = .10). One month after transplant, the tacrolimus dosage and concentration/dosage ratio also showed no significant changes among the 3 groups (P = .09) (Table 2). Clinically acute cellular rejection occurred in 13 patients with CT, 3 homozygous TT, and 2 homozygote CC patients. Although the correlation between these mutations and rejection was not significant (P = .61); however, the C allele was the major allele in rejection group.

Respecting *CYP3A5*, 95% of recipients were harboring a *3/*3 genotype. No obvious difference of *CYP3A5* variants was noticed with tacrolimus dosage adjusted from 1 week to 1 month after transplant (Table 3). All patients with rejection had a *3/*3 genotype.

Data from the present study showed that no statistically significant association was observed between tacrolimus daily dosage (P = .11), tacrolimus dosage adjusted (P = .19), and polymorphisms of the above-mentioned genes (P = .20).

Table 2. Distribution of CYP3A5 and ABCB1 genotypes with allele frequency in transplant recipients.

	Genotype (%)			Allele (%)	
CYP3A5 gene	*3/*3	*1/*3	*1/*1	*3	*1
	95 (95)	5 (5)	0	195 (97.5)	5 (2.5)
	Genotype (%)			Allele (%)	
ABCB1 gene	TT	CT	CC	T	C
	33 (33)	51 (51)	16 (16)	117 (58.5)	83 (41.5)

Abbreviations: ASCT, autologous stem cell transplant; CR, complete remission

Discussion

Tacrolimus, a calcineurin inhibitor, is a potent immunosuppressant to prevent allograft rejection. However, it has a narrow therapeutic window and is highly variable and has unpredictable pharmacokinetics in individual patients. Therapeutic drug monitoring adjusts immunosuppressants to optimize the efficacy and limit toxicity of these drugs. Various pharmacogenetics and pharmacogenomics ethical factors may influence calcineurin inhibitor metabolism. Tacrolimus is metabolized by the CYP enzyme system in the intestine and liver.[12, 13] The drug also is a substrate of P-gp, which is encoded by the MDR1 gene. In the intestine, biliary tract, and kidney, it can decrease the absorption or accelerate excretion of drugs.[18]

Another factor that affects drug level, especially in early stage after transplant, is steroid as pulse therapy. Shimada and associates found that in a living-donor liver transplant patient who received tacrolimus with pulse of steroid for 3 days, the blood concentration of tacrolimus decreased, followed by a gradual recovery to presteroid levels within 2 weeks.[19]

They suggested that the decrease in the blood tacrolimus concentration caused by high-dose steroid therapy is a consequence of the induction of P-gp and CYP3A in the liver and the intestine, and these changes were reversed within 2 weeks after stopping steroid treatment.[19]

The frequency of the CYP3A5 6986A>G SNP is reported to be highly dependent on ethnicity. The CYP3A5*1 allele (CYP3A5 expression) is present in 5% to 15% of whites, 45% to 73% of African Americans, 15% to 35% of Asians, and 25% of Mexicans.[20] In our study, the allelic frequencies of *CYP3A5* *1 and *3 were 2.5% and 97.5%, which was different from above-mentioned ethnicities.[21, 22]

The tacrolimus C0/dosage and doubling of tacrolimus dosage requirements in CYP3A5 expressors was different when they compared them with CYP3A5 nonexpressor individuals.[15, 16]

Table 3. Association of CYP3A5*3 and ABCB1 SNPs with tacrolimus level in transplant recipients.

CYP3A5 genotype	7 days			1 month		
	T0 (ng/mL)	Daily dosage (mg/kg/d)	Dosage-adjusted T0 (ng/mL/mg/kg/d)	T0 (ng/mL)	Daily dosage (mg/kg/d)	Dosage-adjusted T0 (ng/mL/mg/kg/d)
*3/*3	5.9 ± 3.2	153 ± 0.015	32.69 ± 9.1	9.9 ± 3.4	0.174 ± 0.014	38.19 ± 3.1
*1/*3	5.0 ± 2.6	0.173 ± 0.018	30.19 ± 3.1	8.9 ± 3.5	0.193 ± 0.021	36.21 ± 1.1
ABCB1 genotype						
TT	5.1 ± 4.2	0.163 ± 0.015	31.29 ± 9.1	8.8 ± 3.4	0.184 ± 0.014	35.19 ± 4.
CT	5.2 ± 3.3	0.167 ± 0.018	31.19 ± 4.1	9.2 ± 2.5	0.193 ± 0.021	6.21 ± 3.1
CC	4.9 ± 3.6	0.171 ± 0.018	29.19 ± 3.1	7.9 ± 3.5	0.185 ± 0.021	37.01 ± 2.1

It is reported that black and other nonwhite recipients clinically require more drugs than do white recipients, because most of the former carry the wild allele of CYP3A5*1. In contrast to blacks, about 80% of whites express the mutant homozygote of CYP3A5*3, with less metabolism of tacrolimus. In an Asian population, 50% express CYP3A5*1/*3 or *1/*1, and another 50% express *3/*3, which is different from either black or white populations.[23-27]

Influence of the ABCB1 3435C>T, 1236C>T, and 2677G>T/A SNPs on the pharmacokinetics of tacrolimus remains doubtful. The majority of studies did not find an association between the ABCB1 3435C>T SNP and tacrolimus pharmacokinetics.[28-32]

In this study, we failed to demonstrate that interindividual variation in tacrolimus daily dosage requirements with *ABCB1* and *CYP3A5* gene polymorphisms in Iranian liver transplant recipients. However, it should be mentioned that liver donor genotype influences tacrolimus pharmacokinetics far more than do the genotypes of the recipient with regard to expression of CYP3A5.

No significant correlation between these mutations and rejection was noticed in our project; however, the 3435C allele was the major allele in rejection group. A reduction in intestinal P-gp expression was observed in subjects who were homozygous for the 3435T allele, and less drug export from the enterocyte was seen. Recipients with 3435C allele seem to absorb less drug, and therefore, may be more susceptible to rejection owing to immunosuppressive underexposure.

Provenzani and associates investigated the effects of CYP3A5 and ABCB1 SNPs in both donors and recipients on tacrolimus blood levels in white liver transplant recipients. They found that tacrolimus dosage requirements were significantly higher in patients receiving a liver with 1 copy of the *1 allele compared to those homozygous for the *3 allele. Respecting recipients' genotypes, the presence of one *1 copy also increased tacrolimus dosages. In respect to the ABCB1 SNPs, no influence on tacrolimus dosing requirements was detected.[33] In Korean people, recipients with the CYP3A5*3 alleles showed higher blood tacrolimus concentrations than did patients with wild-type alleles, and they emphasized that the CYP3A5 genotype of the liver transplant is important and affects tacrolimus concentrations.[34] Another study from an Iranian population reported no correlation between a CYP3A5 gene polymorphism and cyclosporine dosage (calcineurin inhibitor) in renal transplant recipients.[35] However, Wang and associates found that CYP3A5 genotype is the most significant genetic factor that affects tacrolimus dosage in renal transplant patients.[36]

Barrera-Pulido and associates found that in Spanish liver transplant patients, presence of the A allele for the CYP3A5 gene was related to greater requirements of tacrolimus in the early days after transplant.[37] In Chinese liver transplant patients, Weilin and associates, suggested that the recipients' ABCB1 and the donors' CYP3A5 genotype affect the tacrolimus dosage requirements. The ABCB1 C3435T polymorphism is a major determinant of tacrolimus trough concentration in Chinese liver transplant recipients, and recipients with 3435CC genotype will require higher dosages of tacrolimus.[38] In a recently published review article by Staatz and associates, a strong association between the CYP3A5 6986A>G SNP and tacrolimus pharmacokinetics was emphasized; however, there is no evidence of organ rejection as a result of genotype-related under-immunosuppression. The ABCB1 SNPs have no clear influence on tacrolimus pharmacodynamics, with conflicting results regarding acute rejection.[5, 6] They claimed that low patient numbers in studies might account for many inconsistent results to date. The majority of studies have evaluated only the effects of individual SNPs; however, multiple polymorphisms and haplotype analyses may be useful, particularly ones that consider both donor and recipient genotypes.[5, 6]

In conclusion, the liver donor genotype influences tacrolimus pharmacokinetics regarding expression of CYP3A5 far more than the genotype of the recipient. Therefore, a study on genetic polymorphism of donors is necessary.

Respecting the ABCB1 gene, although 3435C>T is a silent polymorphism that does not result in an amino acid change in the encoded protein, it may be in linkage disequilibrium with other functional polymorphisms of the ABCB1 gene, including 2677G>T/A, which causes a serine-alanine substitution and results in low expression of intestinal P-gp. For a more-comprehensive picture, it is also advisable to investigate the G2677TT/A polymorphism. A larger clinical study including both donor and recipients is needed to evaluate the relation between the pharmacokinetics and pharmacodynamics of tacrolimus metabolism in our population.

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