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Effect of MDR1 Polymorphisms on the Blood Concentrations of Tacrolimus in Turkish Renal Transplant Patients

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ABSTRACT

Background. Tacrolimus, a calcineurin inhibitör, is prescribed to prevent allograft rejection in renal transplantation. Tacrolimus not only has a narrow therapeutic index, but also shows significant interindividual differences. The absorption and metabolism of this drug are affected by multidrug resistance (MDR) 1 gene polymorphisms that correlated with single-nucleotide polymorphisms (SNPs) affecting in vivo P-glycoprotein activity. This study investigated associations of MDR1 gene C3435T polymorphism with tacrolimus blood concentrations and dose requirements as well as acute rejection episodes among Turkish renal transplant patients.

Methods. One hundred living-donor transplant recipients and 150 healthy control subjects underwent C3435T genotyping using polymerase chain reaction–restriction fragment length polymorphism. Blood concentrations of tacrolimus were determined with the cloned enzyme donor immunoassay.

Results. The CC, CT, and TT genotype frequencies among patients were, respectively, 44.0%, 33.0%, and 23.0% versus 36.7%, 43.3%, and 20.0% among control subjects. There was no significant difference between ($P = .061$; $P = .102$; $P = .211$; respectively). The ratio of blood concentration to dose of tacrolimus for patients with mutant homozygous 3435 TT genotype was higher than that of wild-type 3435 CC genotype homozygous individuals. The doses for these patients were lower at 1, 3, and 12 months ($P = .048$; $P = .03$; $P = .041$, respectively). There were no significant differences between the groups regarding coprescription of drugs that affect tacrolimus concentrations, such as diltiazem. Acute rejection episodes were not associated with the CC vs CT or TT genotypes: odds ratio (OR), 0.517 (95% confidence interval [CI], 0.190–1.407; $P = .192$); OR 1.558 (95% CI, 0.587–4.136; $P = .372$); OR 1.346; (95% CI, 0.456–3.968; $P = .590$), respectively.

Conclusions. Determination of MDR1 polymorphism may help to achieve target of tacrolimus blood concentrations.

The calcineurin inhibitor tacrolimus a primary immunosuppressive drug to prevent allograft rejection in transplant patients, is a macrolide first isolated from *Streptomyces tsukubaensis*.[1] It binds to immunophilins known as FK-binding proteins, generating a complex that interferes with the activity of a critical phosphatase, calcineurin.[2–4] It has a narrow therapeutic index, requiring monitoring of trough blood concentrations during chronic therapy. Despite efforts to individualize tacrolimus therapy, a large percentage of patients suffer adverse effects, especially nephrotoxicity.[3,4]

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The oral drug bioavailability is quite variable. Interindividual differences in tacrolimus pharmacokinetics relate at least in part to multidrug resistance (MDR) 1 gene polymorphisms.[5] The human MDR1 gene encodes a 170-kDa transmembrane glycoprotein, (P-glycoprotein (P-gp), which belongs to the ATP-binding cassette superfamily.[6] This protein is composed of 1,280 amino acids with 2 homologous halves containing 6 putative hydrophobic transmembrane segments and an intracellular binding site for ATP.[7] P-gp is reportedly expressed in various normal human tissues, including small and large intestine, adrenal, kidney, liver, and capillary endothelial cells of the brain.[8] Human P-gp is encoded by the MDR1 gene, which is located on chromosomal region 7q21 consisting of 28 exons.[9] P-gp is an important ATP-dependent membrane transporter which is involved in the absorption, distribution, and elimination of numerous drugs. It acts as an energy-dependent efflux pump that exports its substrates out of the cell.[10,11] A number of MDR1 gene polymorphisms have been shown to be of clinical importance, because they can alter drug absorption, distribution, and elimination.[12] There is broad substrate specificity of P-gp for generally hydrophobic substances: anticancer agents, antibiotic transporters, immunosuppressants, human immunodeficiency virus (HIV) protease inhibitors, and antihistamines.[13]

Hoffmeyer et al reported a significant correlation between polymorphism in exon 26 (C3435T) of MDR1 with the expression level and function of P-gp. Individuals homozygous for the C3435T allele displayed significantly reduced duodenal MDR associated with increased digoxin (a substrate of P-gp) plasma levels.[14] C3435T polymorphism in exon 26 of the MDR1 gene leads to amino acid changes that a silent polymorphism. The effect of these polymorphisms on P-gp function and their clinical impact is in most cases unknown; some polymorphisms alter the pharmacokinetics of substrate drugs. A single-nucleotide polymorphisms (SNP) at 26 exon C3435T of the MDR1 gene, which decreases the expression of P-gp, causes interindividual variability of expression affecting responses to medications.[15] The calcium channel blocker diltiazem has been used widely as a tacrolimus-sparing agent, because it increases drug blood concentrations.[16] Lansoprazole is also a substrate of P-gp.[17] Therefore, polymorphisms in the MDR1 gene may affect drug interactions between tacrolimus and lansoprazole. Interestingly, polymorphism in exon 26 of the human MDR1 gene (C3435T) has been associated with changes in the expression level and function of intestinal P-gp.[18] In the present study, we investigated the effect of MDR1 C3435T polymorphism on tacrolimus blood concentrations and acute rejection episodes among Turkish renal transplant patients.

MATERIALS AND METHODS

Patient Population and Clinical Data Collection

The subjects included 150 healthy control subjects (89 female and 61 male) and 100 adult renal transplant patients (53 female and 47 male), each of whom provided written informed consent. The median age of the 100 adult renal transplant patients was 35.47 ± 11.58 years (range, 19–64); that of the 150 healthy controls was 40.18 ± 12.15 years (range, 19–65). At 1, 3, and 12 months after renal transplantation are from living donors, we collated data including weight (kg) and daily tacrolimus doses (mg/d) at 1, 3, 6, and 12 months as well as calculated dose per weight (mg/kg/d). Also, we studied, acute rejection episodes and graft function.

Immunosuppressive Regimen

Immunosuppressive therapy combined tacrolimus with mycophenolate mofetil or azatioprine or mycophenolate sodium as a purine inhibitor as well as prednisolone as the steroid. After initial tacrolimus doses of 0.15 mg/kg/d, the target trough concentrations were 10–20 ng/mL during the first 3 months and then 5–15 ng/mL. Dose-adjusted concentrations (ng/mL per mg/kg body weight) were calculated by dividing trough values by the corresponding daily dose. Patients were excluded if they were prescribed a medication that affected tacrolimus blood concentrations; namely, diltiazem, verapamil, rifampin phenytoin, lansoprozole, erythromycin, or clarithromycin. Demographic and clinical characteristics of patients are summarized in Table 1 Seventeen patients experienced biopsy-proven acute rejection episodes after transplantation. Banff 97 working classification criteria were used to evaluate biopsies.[19] This study protocol was approved by our Research Ethics Committee.

DNA Samples and DNA Extraction

Whole blood samples collected in EDTA-containing tubes yielded genomic DNA extracted with the use of a purification kit (Peqlab)

Table 1. Demographics of Renal Transplant Patients

Demographic Characteristics	Value
No. of patients	100
Sex (female/male)	53/47
Age (y, range)	35.47 ± 11.58, 19–64
Transplantation no. (1/2)	100/0
Body weight (kg)	60.57 ± 11.33
Height (cm)	167.76 ± 9.04
Primary kidney disease, n (%)	
Chronic glomerulonephritis	28 (28.0%)
Tubulointerstitial nephritis	25 (25.0%)
Unknown	20 (20.0%)
Primary nephrosclerosis	13 (13.0%)
Amyloidosis	12 (12.0%)
Diabetic nephropathy	2 (2.0%)
Acute rejection episodes, n (%)	22 (22.0%)
Biopsy-proven acute rejection, n (%)	17 (77.2%)
Clinical rejection, n (%)	5 (22.8%)
Antirejection therapy, n (%)	
Steroid	10 (10.0%)
Antithymocyte globulin (ATG)	2 (2.0%)
Steroid + ATG	2 (2.0%)
Steroid + intravenous immunoglobulin (IVIG)	5 (5.0%)
Steroid + ATG + IVIG	3 (3.0%)
Immunosuppressive therapy, n (%)	
Tacrolimus/mycophenolate mofetil/prednisolone	56 (56.0%)
Tacrolimus/mycophenolate sodium/prednisolone	29 (29.0%)
Tacrolimus/azatioprine/prednisolone	15 (15.0%)

according to manufacturer's protocol. DNA concentrations were determined by the 260 and 280 nm values. DNA samples were stored at −20°C until use.

Identification of Genotypes of C3435T Polymorphism

Polymorphisms in C3435T (exon 26) of the MDR1 gene were determined by polymerase chain reaction (PCR)–restriction fragment length polymorphism as described previously.[18] PCR was performed in a total volume of 50 μL, using 50 ng genomic DNA with 100 pmol/μL forward primer (5′TTGATGGCAAAGAAATAAAGC′3) and reverse primer (5′CTTACATTAGGCAGTGACTCG′3), 2 mmol/L dNTP, 10× PZR Buffer (100 mmol/L Tris-HCl, 500 mmol/L KCl, 25 mmol/L $MgCl_2$) and 1.25 Taq DNA polymerase. PCR conditions were: initial denaturation at 94°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 60 seconds and a final extension at 72°C for 5 minutes. PCR products were then submitted to Mbo I digestion at 37°C for 4 hours, which cleaves the 3435C allele to 145 base pair (bp) and 62 bp fragments, whereas 3435T cannot be cleaved, retaining the original length of 207 bp. The heterozygote CT genotype can be cleaved by Mbo I to fragments of 207 bp, 145 bp, and 62 bp. Reaction products were separated on 2% agarose gel electrophoresis.

Tacrolimus Blood Concentration

The blood concentrations of tacrolimus were assayed by the cloned enzyme donor immunoassay method using reagents in the MGC 240 kit (Microgenics).

Statistical Analysis

All descriptive statistics were computed with SPSS version 16 (Chicago, IL, USA). Chi-square and Fisher exact tests were used to evaluate the genotype distribution frequencies. A value of $P < .05$ was considered to be statistically significant. Hardy-Weinberg equilibrium was assessed using the chi-square test. Genotype and allele frequencies are given with their 95% confidence intervals (95% CIs). Daily doses and dose-adjusted tacrolimus blood levels are expressed as mean ± SD. For comparison of genotype groups, Student *t* test was used. Between-group comparisons for MDR1 genotypes were performed by 1-way analysis of variance.

RESULTS

Among the patients, the frequency of the CC genotype was 44.0% (44/100), the CT genotype 33.0% (33/100), and the TT genotype 23.0% (23/100). Among the healthy control subjects, the frequency of the CC genotype was 36.7% (55/150), the CT genotype 43.3% (65/150), and the TT genotype 20.0% (30/150), which was not different from the renal transplant patients: odds ratio [OR] 1.211 (95% CI 0.954–1.545; $P = .061$), OR 0.75 (95% CI 0.550–1.010; $P = .102$), OR 0.500 (95% CI 0.287–0.870; $P = .211$), respectively (Table 2). Genotype distribution of C3435T MDR1 polymorphism showed Hardy-Weinberg equilibrium among both healthy control subjects and patients (*P* values .598 and .664, respectively).

Blood concentrations of tacrolimus were significantly higher among patients with the 3435TT genotype at 1 ($P = .043$) and 12 ($P = .026$) months after transplantation versus subjects of the 3435CT and 3435CC genotypes. Individuals bearing the 3435TT genotype demonstrated lower dose requirements at 1 ($P = .03$), 3 ($P = .041$), and 12 ($P = .048$) months compared with those of the 3435CT and 3435CC genotypes. Table 3 presents significant differences among the genotype groups in regarding concentration/dose ratios at 1, 3, and 12 months (*P* values .0001, .004, and .002, respectively). Significant differences in serum creatinine levels at 1 month were noted between 3435TT genotype versus 3435CC and 3435CT genotypes: namely, 1.41 ± 0.41 mg/dL versus 1.28 ± 0.31 mg/dL and 1.28 ± 0.39 mg/dL (*P* values <.0001 and <.0001, respectively). At 3 months there were significant differences in mean serum creatinine levels between 3435TT genotype versus 3435CC and 3435CT genotypes: namely, 1.41 ± 0.38 mg/dL versus 1.28 ± 0.41 mg/dL and 1.26 ± 0.31 mg/dL (*P* values <.0001 and <.0001, respectively; Table 3).

Similar results were observed among patients on immunosuppressive regimens, including mycophenolate mofetil or azatioprine or mycophenolate sodium as the purine inhibitor ($P > .05$).

The incidence of acute rejection episodes differed among groups of different MDR1 3435 genotypes ($P > .05$; Table 4). The tacrolimus blood concentrations was elevaluated between the groups with different MDR1 genotypes and acute rejection episodes (Table 5).

DISCUSSION

Tacrolimus shows a narrow therapeutic index; thus it is a critical-dose drug requiring therapeutic drug monitoring to avoid nephrotoxicity. Tacrolimus shows marked large inter-

Table 2. Frequency of C3435T SNPs in Healthy Control Subjects and Renal Transplant Patients

	Renal Transplant Patients (n:100)		Healthy Controls Subjects (n:150)			
Genotype	n	%	n	%	OR (95% CI)	*P* Value
3435CC	44	44.0%	55	36.7%	1.357 (0.810–2.274)	.303*
3435CT	33	33.0%	65	43.3%	1.195 (0.646–2.208)	.681†
3435TT	23	23.0%	30	20.0%		
Allele						
C	121	60.5%	175	58.5%	1.094 (0.759–1.576)	.697
T	79	39.5%	125	41.5%		

*CC and TT+CT.
†TT and CC+CT.

Table 3. Pharmacokinetic Parameters of Tacrolimus in Different MDR1 C3435T Genotypes

	3435CC	3435CT	3435TT
No. of patients	44	33	23
Month 1			
Tacrolimus daily dose (mg/kg/d)	0.13 ± 0.08*	0.10 ± 0.05	0.09 ± 0.06
Tacrolimus blood concentration (ng/mL)	15.44 ± 7.29	15.22 ± 6.93	16.92 ± 7.33*
Blood concentration/Dose (ng/mL per mg/kg)	118.76 ± 41.21	152.20 ± 51.12	188.00 ± 35.65*
Serum creatinine (mg/dL)	1.28 ± 0.31	1.28 ± 0.39	1.41 ± 0.41*
Month 3			
Tacrolimus daily dose (mg/kg/d)	0.11 ± 0.10*	0.09 ± 0.04	0.08 ± 0.07
Tacrolimus blood concentration (ng/mL)	11.03 ± 4.56	10.97 ± 4.82	11.13 ± 4.52
Blood concentration/dose (ng/mL per mg/kg)	100.27 ± 35.21	121.88 ± 53.03	139.12 ± 48.24*
Serum creatinine (mg/dL)	1.28 ± 0.41	1.26 ± 0.31	1.41 ± 0.38*
Month 6			
Tacrolimus daily dose (mg/kg/d)	0.09 ± 0.07	0.08 ± 0.07	0.08 ± 0.06
Tacrolimus blood concentration (ng/mL)	8.65 ± 3.63	9.12 ± 4.21	9.41 ± 3.98
Blood concentration/dose (ng/mL per mg/kg)	96.11 ± 22.12	114.00 ± 28.56	117.62 ± 23.65
Serum creatinine (mg/dL)	1.26 ± 0.32	1.24 ± 0.30	1.38 ± 0.43
Month 12			
Tacrolimus daily dose (mg/kg/d)	0.09 ± 0.07*	0.07 ± 0.05	0.07 ± 0.04
Tacrolimus blood concentration (ng/mL)	7.39 ± 2.98	6.65 ± 2.21	8.70 ± 3.35*
Blood concentration/Dose (ng/mL per mg/kg)	82.11 ± 35.16	95.00 ± 39.56	124.28 ± 48.41*
Serum creatinine (mg/dL)	1.21 ± 0.26	1.20 ± 0.34	1.21 ± 0.34

Data are presented as mean ± SD.
*$P < .05$.

and intraindividual variabilities in blood concentrations despite fixed doses.[20,21] 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 drug absorption or accelerate their excretion. Tacrolimus is a substrate for P-gp, which may act as a barrier to oral drug absorption. Its functional and expressonal variation affects the bioavaliability of tacrolimus.[22] MDR1 gene polymorphism of C3435T has shown a relationship with tacrolimus dose requirements.[20,23–25] Comparison of the polymorphism genotype frequencies of C3435T between healthy control subjects and renal transplant patients did not show a significant difference in a Chinese population.[26] Kotrych et al also reported no significant differences in the frequency of MDR1 C34345T genotypes between kidney transplant patients and a healthy population.[22] In the present study, no significant difference was observed between the C3435T genotype frequencies in Turkish healthy control subjects and renal transplant patients ($P > .05$).

Genetic variability may affect drug pharmacokinetics. Masuda et al described an association between low bioavailability of tacrolimus in renal transplant patients and high P-gp expression in the gastrointestinal system.[27] Several studies have associated MDR1 gene polymorphism with tacrolimus dosages. However, conflicting results have been reported. Hesselink et al failed to observe a significant difference between MDR1 C3435T polymorphism and tacrolimus doses,[24] Macphee et al reported only a weak association,[25] and Tada et al showed no effect on tacrolimus pharmacokinetics.[28] In contrast in a study of 92 Turkish renal transplant recipients, Akbas et al noted tacrolimus daily doses to be significantly lower at 1 and 6 months after transplantation among patients with the 3435TT genotype.[29]

Association of the MDR1 gene with tacrolimus dose requirements has been recognized as a genetic basis for observed inter individual differences in pharmacokinetics.[30] Our results also showed polymorphism of C3435T to influence pharmacokinetic paremeters at different posttransplantation times among Turkish renal transplant patients. Patients of the CC genotype showed lower blood concentration dose/ratios and a requirement for higher tacrolimus doses than those of the CT or TT genotypes at 1, 3, and 12 months after transplantation. Akbas et al reported no significant differences in serum creatinine levels among MDR1 C3435T genotypes.[29] In contrast, the present study demonstrated an association between MDR1 C3435T polymorphism and serum creatinine values, which were significantly higher at 1 and 3 months after transplantation among patients with the 3435TT genotype. Most patients

Table 4. Distribution of MDR1 3435 Genotypes in Patients With Rejection and Without Rejection, n (%)

Genotype	Patients With Rejection	Patients Without Rejection	OR (95% CI)	*P* Value
3435CC (n = 44)	9 (40.9%)	35 (44.9%)	0.517 (0.190–1.407)	.192
3435CT (n = 33)	7 (31.8%)	26 (33.3%)	1.558 (0.587–4.136)	.372
3435TT (n = 23)	6 (27.3%)	17 (21.8%)	1.346 (0.456–3.968)	.590

Table 5. The Blood Concentrations of MDR1 3435 Genotypes in Patients With Rejection and Without Rejection

	Tacrolimus Blood Concentration (mg/mL)		
	Patients With Rejection	Patients Without Rejection	*P* Value
Month 1			
CC	13.23 ± 6.89	14.78 ± 6.95	.789
CT	12.76 ± 5.24	13.47 ± 4.21	.541
TT	13.45 ± 6.49	15.98 ± 7.31	.102
P Value	0.532	0.487	
Month 3			
CC	6.23 ± 7.25*	9.45 ± 6.43*	.045
CT	8.21 ± 6.03	9.22 ± 5.14	.689
TT	10.24 ± 7.13*	10.03 ± 6.47	.946
P Value	0.024	0.519	
Month 6			
CC	6.13 ± 4.65*	7.99 ± 6.03*	.445
CT	7.97 ± 5.46	8.27 ± 6.45	.913
TT	7.45 ± 6.98	8.11 ± 4.69	.769
P Value	0.261	0.881	
Month 12			
CC	5.28 ± 5.46*	8.03 ± 65.47	.031
CT	6.97 ± 6.39	9.46 ± 6.04	.094
TT	7.12 ± 4.97	8.44 ± 5.02	.213
P Value	0.018	0.881	

Data are presented as mean ± SD.
*$P < .05$.

did not reach target concentrations using the recommended initial doses of tacrolimus. Therefore patients showed an increased risk of underimmunosuppression and acute rejection episodes.[25] Among liver transplant patients on tacrolimus treatment, Rahsaz et al failed to observe MDR1 C3435T genotypes to be associated with rejection episodes.[31] Also, Rahsaz et al reported the 3435C allele to be the major allele among the rejection group. In our study, we also demonstrated that MDR1 C3435T polymorphism did not affect acute rejection episodes.

A reduction in intestinal P-gp expression has been observed among subjects who are homozygous for the 3435T allele. These recipients seem to absorb less drug and therefore may be more susceptible to a rejection process owing to immunosuppressive underexposure. In contrast, in a retrospective study, Li et al noted that when coadministered with diltiazem, the mean increments in dose-adjusted blood levels for tacrolimus were larger among CYP3A5 expressors than CYP3A5 nonexpressors.[16] In the present study, we excluded patients taking medication that affected tacrolimus blood concentrations, such as diltiazem.

In conclusion, therapeutic drug monitoring of tacrolimus has an important role to avoid nephrotoxicity and acute rejection episodes. Our results demonstrated a correlation between the C3435T polymorphism of MDR1 gene and tacrolimus pharmacokinetics among Turkish renal transplant patients. MDR1 gene polymorphism may be helpful to individualize tacrolimus treatment of renal transplant patients. (Table 5).

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