

Association between Cyclosporine Concentration and Genetic Polymorphisms of CYP3A5 and MDR1 during the Early Stage after Renal Transplantation

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Objectives: Cyclosporine (CsA) has a narrow therapeutic range, and its pharmacokinetic characteristics vary among individuals. It also is a substrate for cytochrome P450 (CYP) 3A and P-glycoprotein, the product of the multidrug resistance 1 (MDR1) and CYP3A5 genes. Some of the single nucleotide polymorphisms (SNPs) in these genes are associated with deficient protein expression and reduced in vivo activity. We postulated that in renal transplant recipients, these SNPs should be associated with interindividual variations in CsA pharmacokinetics.

Materials and Methods: In 88 Iranian renal transplant patients receiving CsA, CYP3A5 and MDR1 genotypes were determined by polymerase chain reaction, followed by restriction fragment length polymorphism analysis. Whole blood trough CsA concentrations were measured by radioactive immunosorbent assay. The dose-adjusted concentration (ng/mL per mg/kg/d) was calculated at 1 day (± 2 days), 7 days, and 1 month after transplantation.

Results: The MDR-1 wild-type genotype (3435CC) was observed in 17 patients (19%), whereas 45 patients (51%) were heterozygous (3435CT), and 26 patients (30%) were homozygous (3435 TT) for the mutation. In the days immediately after transplantation, we found a correlation between the concentration/dose ratio and the exon 26 MDR single nucleotide polymorphisms ($33.3 \pm 15.24 \mu\text{g}$

mg/L/kg in the CT group vs $44.1 \pm 28.4 \mu\text{g mg/L/kg}$ in the TT group, $P = .019$). This ratio was significantly higher in subjects homozygous for the mutation (3435TT). This significant difference was not seen 1 week or 1 month after transplantation. All patients had the CYP3A5*3/*3 genotype, so no differences among the CYP3A5*1/*3 genotypes were found.

Conclusions: MDR-1 (3435CC) polymorphisms are associated with CsA pharmacokinetics and dose requirements in the first few days after renal transplantation. Pharmacogenetic methods could be used to help select the initial dosage and individualize immunosuppressive therapy. According to our results, the major genotype of our recipients is CYP3A5*3/*3. According to the literature, the recommended starting dosage of CsA is 9-14 mg/kg/day; however, the Iranian population has a good response with lower dosages (3-5 mg/kg/day), which may be explained by genetic differences.

Key words: Cyclosporine, MDR1, Cyp3A5, Polymorphism, Kidney transplantation

Cyclosporine (CsA) is a calcineurin inhibitor that prevents allograft rejection after solid organ transplantation. It is characterized by a narrow therapeutic index. Its pharmacokinetic characteristics vary greatly among individuals, and daily dosages must be adjusted to maintain the desired whole-blood CsA concentration [1]. CsA is known to be a substrate of cytochrome P450 (CYP) 3A5 and P-glycoprotein (P-gp), which are encoded by CYP3A5 and multidrug resistance 1 (MDR1) genes, respectively [2, 3]. Differences in expression levels and bioactivity of these proteins may explain interindividual variations in CsA pharmacokinetics [4, 5]. Four CYP3A isoenzymes have been identified: CYP3A4, CYP3A5, CYP3A7, and CYP3A43, and

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among these, CYP3A4 and CYP3A5 are the major components [4]. CYP3A5 is expressed heterogeneously among different populations and accounts for a great part of total CYP3A enzyme content in the intestine and the liver. A frequent single nucleotide polymorphism (SNP) 6896A>G has been found to be associated with CYP3A5 protein production and enzyme activity [4]. The G>A mutation in intron 3 results in a splice defect of the mRNA and produces an unstable and nonfunctional protein. The mutated allele was named CYP3A5*3, and the wild-type was assigned CYP3A5*1. Only individuals carrying at least one CYP3A5*1 allele can express high levels of the CYP3A5 enzyme [4, 5, 6]. P-gp is a product of the multidrug resistance gene (MDR1, called ABCB1) and acts as a transmembrane efflux pump involved in energy-dependent export of xenobiotics from inside to outside the plasma membrane [7]. It is present in intestinal epithelial cells and biliary canalicular cells, and is expressed in the blood-brain barrier, lymphocytes, and on the luminal surface of proximal tubule kidney cells [8]. Therefore, P-gp may affect absorption of drugs from the gut, their distribution in the body, and their excretion [8, 9].

Several SNPs have been described in the MDR1 gene. One of them, the SNP of the C to T exchange at nucleotide 3435 located in exon 26, has been shown to be associated with lower intestinal P-gp expression and activity in vivo [8]. Individuals homozygous for the mutation at position 3435 (TT) have significantly lower P-gp levels in the small intestine compared with the remainder of the population [8, 9]. In this study, we investigated the association of CYP3A5 and MDR1 genotypes in 88 de novo renal transplant recipients and established whether this polymorphism was associated with CsA dosage requirements and concentration/dosage ratio in achieving the target therapeutic range in blood (180 ng/mL) 1 month after transplantation.

Materials and Methods

A total of 88 renal transplant recipients (56 males, 32 females) who underwent transplantation at the Transplantation Center of Nemazi Hospital in Shiraz, Iran, were included. The average age was 36.92 ± 13.16 years (range, 13-73 years). The average body weight was 57.31 ± 14 kg. All patients received compound immunosuppressive therapy consisting of steroids, CsA, and either mycophenolate mofetil

or azathioprine. The steroid regimen was 500 mg IV methylprednisolone at the time of surgery and then 1 g/day IV each day for the next 3 days. This schedule was followed by 30 mg/day of oral prednisolone, progressively tapered to 15-20 mg/day at the end of 1 month. The initial dosage of CsA was 3-5 mg/kg/day, which was begun during surgery. The daily dosage was then adjusted, according to blood trough CsA concentration (C_0), to a target concentration of 180 ng/mL. Mycophenolate mofetil was given 1 g b.i.d., and azathioprine was administered at 2.5 mg/kg/day as single daily dose during the first month after surgery. These dosages were sometimes changed after 1 month according to clinical and laboratory findings of the patients. Body weights, CsA dosages, and whole blood concentrations were recorded at 1 day (± 2 days), 7 days, and 1 month after transplantation. The weight-adjusted CsA dosage (mg/kg/d) and the dose-adjusted C_0 (ng/mL per mg/kg/d) were calculated. Patients received no other drugs known to interact with CsA. Acute clinical rejection was recorded, and when it occurred, steroid pulse therapy was started. If the patient had no clinical response, a kidney biopsy was performed. Biopsy specimens were graded according to Banff criteria [10].

The study was performed in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Shiraz University of Medical Sciences. Written informed consent was obtained from all subjects.

CsA dosage was given daily, in equal amounts, at 8:00 AM and 8:00 PM. To determine C_0 , blood samples (using ethylenediaminetetraacetic acid as an anticoagulant) were collected at 8:00 AM, just before the morning dose. CsA levels were measured in the whole blood using the radioimmunoassay method (DiaSorin, Stillwater, Minn, USA). Data were recorded at 1 day (± 2 days), 7 days, and 1 month after transplantation. To determine the genotype, DNA was extracted from a peripheral blood sample using a commercial extraction kit and stored at -20°C until analysis. A polymerase chain reaction (PCR)-restriction fragment length polymorphism was used to detect C3435T and CYP3A5 SNP, according to the methods described by Ameyaw and colleagues [11] and Tsuchiya and colleagues [12]. Samples were digested with restriction enzymes, MboI (Roche Diagnostics Corp., Penzberg, Germany) for MDR1, and SspI (Roche Diagnostics Corp., Penzberg, Germany) for Cyp3A genes, and then were

separated on a 2.5% agarose gel. Data are expressed as means $\pm$ SD. Analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 11.5, SSPS Inc, Chicago, Ill, 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. A one-way ANOVA, followed by the Dunn test for multiple comparisons, also was performed.

Results

Patient demographics are shown in Table 1. No significant differences regarding age or weight were observed. Of the 88 kidney transplant recipients, the MDR wild-type genotype (3435CC) was observed in 17 patients (19%), whereas 45 patients (51%) were heterozygous (3435CT), and 26 patients (30%) were homozygous (3435 TT) for the mutation (Table 2). In the first days after transplantation (days 1-3), patients received similar dosages of CsA, and the mean weight-adjusted CsA dose was not significantly different among the 3 groups. However, we did find a correlation between the concentration/dose ratio and exon 26 SNP ($33.3 \pm 15.24 \mu\text{g mg/L/kg}$ in the CT group and $44.1 \pm 28.4 \mu\text{g mg/L/kg}$ in the TT group, *P* = .019). This ratio was significantly higher in subjects homozygous for the 3435TT mutation (Table 2). In the 3 groups, the CsA dosage was increased at day 7 to reach the target therapeutic C_0 level of $180 \mu\text{g/L}$. After 1 week of adjusted doses, the mean CsA C_0 level reached the target therapeutic range, and no significant differences were observed among the 3 groups. One month after transplantation, the CsA dosage and concentration/dose ratio also showed no significant changes among the 3 groups. Biopsy-confirmed acute cellular rejection occurred in 2 patients with wild-type mutations and in 2 homozygous (3435 TT) patients. The correlation between these mutations and rejection was not significant (*P* = .31). All 88 recipients had the CYP3A5 *3/*3 genotype, which was confirmed by sequencing the products.

Table 1. Characteristics of 88 renal transplant recipients according to MDR1 3435 genotype

MDR1 3435 genotype	CC	TT	CT
n (%)	17 (19.3)	26 (29.5)	45 (51.1)
Mean age (years $\pm$ SD)	30.94 $\pm$ 9.3	35.27 $\pm$ 12.07	40.13 $\pm$ 4.23
Sex (F/M)	10 / 7	9 / 17	13 / 32
Mean weight (kg $\pm$ SD)	58.59 $\pm$ 14.41	57.31 $\pm$ 14.06	56.51 $\pm$ 13.03

MDR 1, multidrug resistance 1

Discussion

Calcineurin inhibitors and especially CsA are widely used P-gp substrates characterized by high interindividual variations in oral bioavailability and a narrow therapeutic index. The frequency of appearance of the 3 genotypes (CC, 19%; CT, 45%; TT, 26%) in the studied population was different from the frequencies reported for the polymorphism in the white (Spanish) population (CC, 34%; CT, 48%; TT, 18%) [13]. We showed that at 1 month, when the CsA dosage was adjusted to the therapeutic C_0 level of $180 \mu\text{g/L}$, the required dosage was not different in patients homozygous for 3435T compared with those with wild-type alleles. In this study, the target concentration of CsA was reached by day 7. We then noted that the required CsA dosage did not change between day 7 and day 30 to maintain the same C_0 target. With regard to the CsA concentration/dose ratio, our results demonstrate that it correlated with MDR1 exon 26 SNP during posttransplantation days 1-3, and that it was higher in subjects homozygous for the mutation. Because the MDR1 3435 TT homozygotes would initially require lower dosages of CsA, MDR1 genotyping could provide useful information for individualizing CsA dosage in renal-transplant recipients at the beginning of treatment, while improving effectiveness and decreasing adverse effects. However, replication of these results in a larger population is warranted.

CYP3A5 is expressed in few individuals. The absence of CYP3A5 expression is reported in 73% of Chinese and 85%-95% of white persons [4]. The CYP3A5 *3 allele results in the loss of hepatic CYP3A5 activity [4]. This indicates that persons

Table 2. Comparison of cyclosporine levels and MDR1 3435 genotypes in 88 renal transplant recipients

MDR1 3435 genotype	MDR1 3435 CC	MDR1 3435 TT	MDR1 3435 CT
CsA 24-h dose/body weight (mg/kg)			
Day 1-3	4.25 $\pm$ 1.3	4.46 $\pm$ 1.2	4.55 $\pm$ 0.91
Day 7	5.09 $\pm$ 1.1	5.15 $\pm$ 1.16	4.66 $\pm$ 1.11
Day 30	5.03 $\pm$ 1.4	5.19 $\pm$ 1.3	4.5 $\pm$ 1.19
Mean CsA C2 level ($\mu\text{g/L}$)			
Day 1-3	111.24 $\pm$ 59.4	172.15 $\pm$ 97.7	149.58 $\pm$ 70
Day 7	156.35 $\pm$ 46.8	190.15 $\pm$ 71.28	189.89 $\pm$ 78.1
Day 30	173.6 $\pm$ 55.9	215.85 $\pm$ 92.6	203.09 $\pm$ 85.5
C_0 level /24-h dose/body weight ($\mu\text{g} \times \text{mg/L/kg}$)			
Day 1-3	26.57 $\pm$ 12.62	44.14 $\pm$ 28.48	33.30 $\pm$ 15.24
Day 7	33.07 $\pm$ 15.88	39.13 $\pm$ 18.80	33.30 $\pm$ 15.24
Day 30	32.66 $\pm$ 10.69	45.23 $\pm$ 24.20	44.53 $\pm$ 23.40

MDR 1, multidrug resistance 1; CsA, cyclosporine; C_0 , blood trough cyclosporine concentration

with CYP3A5 *1/*3 expression require more CsA to achieve the same target blood concentration than do those with CYP3A5 *3/*3 expression. In this study, we failed to demonstrate an association between CsA dosage requirement and the CYP3A5 polymorphism. However, the Iranian population, with predominant CYP3A5 *3/*3 expression, needs less CsA to achieve the desired blood concentration.

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