Polymorphism of the CYP3A5 Gene and Its Effect on Tacrolimus Blood Level

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Abstract

Objectives: Tacrolimus is the cornerstone for immunosuppression in renal transplant and is metabolized by the cytochrome P 450 3A (CYP3A) subfamily of enzymes in the liver and small intestine. A polymorphism in intron 3 of the CYP3A5 gene affects the expression of this enzyme and tacrolimus trough blood levels. The purpose of this study was to identify the proportion of CYP3A5 gene polymorphisms in South Indian renal transplant patients and determine the effect of CYP3A5 gene polymorphisms on tacrolimus trough blood levels in patients with and without CYP3A5 expression.

Materials and Methods: We included 25 adult patients who underwent renal transplant at Government Medical College, Trivandrum. All patients received tacrolimus (dose, 0.1 mg/kg/body weight, in 2 divided doses). Tacrolimus trough blood levels were determined on postoperative day 6. The CYP3A5 genotype analysis was performed by polymerase chain reaction amplification of target and detection by restriction fragment length polymorphism analysis.

Results: The CYP3A5*1/*1 genotype was detected in 5 recipients (20%), *1/*3 genotype in 5 recipients (20%), and *3/*3 genotypes in 15 recipients (60%) of the total 25 graft recipients.

Mean tacrolimus level in the CYP3A5*1/*1 group was 5.154 ng/mL (range, 4.42 to 6.5 ng/mL), CYP3A5*1/*3 group was 5.348 ng/mL (range, 3.1 to 9.87 ng/mL), and CYP3A5*3/*3 group was 9.483 ng/mL (range, 4.5 to 14.1 ng/mL). Acute rejection episodes were significantly more frequent for CYP3A5*1/*1 homozygous patients (40%) than patients with CYP3A5*1/*3 (20%) or CYP3A5*3/*3 (13%) genotypes.

Conclusions: Most patients carried the mutant allele CYP3A5*3 (A6986G). Tacrolimus drug level correlated well with presence or absence of CYP3A5 polymorphisms. Acute rejection episodes were more frequent in expressors, and they may require higher doses of tacrolimus. Similarly, tacrolimus nephrotoxicity was more frequent in non-expressors. Therefore, CYP3A5 polymorphism analysis before renal transplant may help determine the optimal dose of tacrolimus in this population and prevent acute rejection episodes or tacrolimus toxicity.

Key words: Cytochrome P450, End-stage renal disease, Genetics, Immunosuppression

Introduction

Tacrolimus is the corner stone of immunosuppression in renal transplant. It is a macrolide antibiotic compound that acts by inhibiting the calcineurin pathway by binding to FK binding protein. However, it has a narrow therapeutic index and requires therapeutic drug monitoring to prevent graft rejection as a result of inadequate immunosuppression with low drug levels or toxicity due to high drug levels.

Tacrolimus is metabolized by the CYP3A subfamily of enzymes in the liver and small intestine.
Although both CYP3A4 and CYP3A5 are involved in the metabolism of tacrolimus, previous studies have shown that polymorphisms in CYP3A5 genes are responsible for interindividual variations in bioavailability of tacrolimus.2

A polymorphism in intron 3 of the CYP3A5 gene affects the expression of this enzyme. The CYP3A5*3 allele (guanine at position 6986) produces a cryptic splice site and encodes an abnormal spliced mRNA with a premature stop codon; thus, individuals who are homozygous for this allele (CYP3A5*3/*3) are called nonexpressors. Presence of CYP3A5*1 allele (adenine at position 6986) produces normal mRNA, resulting in a high expression of this enzyme in the intestine and in the liver; individuals expressing at least one CYP3A5*1 allele are called expressors. Therefore, expressors can be either homozygous (CYP3A5*1/*1) or heterozygous (CYP3A5*1/*3).3,4

Previous studies showed that expressors achieved 2-fold lower tacrolimus concentration-to-dose ratio compared with nonexpressors.5-9 Therefore, we aimed to find the proportion of nonexpressors and expressors and clarify the role of CYP3A5 polymorphism on tacrolimus drug levels in our renal transplant population.

**Materials and Methods**

**Patients**

We included 25 adult patients who underwent renal transplant at Government Medical College, Trivandrum, Kerala, India. The study was approved by the ethics committee of the institution before the study began, and the protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all patients. All patients received tacrolimus (dose, 0.1 mg/kg bodyweight) with prednisolone and mycophenolate mofetil. Tacrolimus 12-hour trough blood level was determined on postoperative day 6 using a chromatographic method (liquid chromatography-tandem mass spectrometry assay). The lower limit of quantification of the assay was 0.003 ng/mL.

**Genotype analyses**

Genotype analyses of all patients were performed to identify the CYP3A5 allele. Nucleic acid isolation was performed using a standard magnetic bead-based extraction protocol (MagJET DNA and RNA Purification Kits, Thermo Fisher Scientific Corporation, MA, USA) according to the manufacturer’s instructions. Custom-designed primers synthesized from human cytochrome P450 PCN3 mRNA (cytochrome P450, family 3, subfamily A, polypeptide 5) complete cDNAs were used for polymerase chain reaction amplification of the target. The polymerase chain reaction protocol was a standardized procedure for the selected primer sequence done using thermal cycler (Applied Biosystem, Thermo Fisher Scientific Corporation. MA, USA). Postamplification detection was performed by restriction fragment length polymorphism analysis using SspI endonuclease (New England Biolabs Inc, MA, USA).

**Statistical analyses**

Data were reported as mean ± standard deviation (SD) or mean (range, minimum to maximum) for all quantitative estimates. Tacrolimus trough blood levels between 2 groups were compared with independent t test. Statistical analysis was done using software (SPSS for Windows, Version 16.0, SPSS Inc., Armonk, NY, USA).

**Results**

**Characteristics of study population**

In the 25 renal graft recipients who were included in the study, there were 22 males and 3 females (Table 1). The mean age was 32 ± 20 years and body weight was 58 ± 11 kg. There were 3 patients who received induction therapy (2 patients received antithymocyte globulin and 1 patient received basiliximab). Mean donor age was 44 ± 12 years. At 1 month after transplant, the incidence of biopsy-proven acute rejection was 20% (all T-cell-mediated rejection). There were 8 graft biopsies obtained from the study population in the first month after transplant, and the incidence of tacrolimus nephrotoxicity in this subgroup was 8%.

**Frequency of CYP3A5 genotypes and relation to tacrolimus level**

The CYP3A5*1/*1, *1/*3, and *3/*3 genotypes were detected in 5 (20%), 5 (20%), and 15 (60%) of the 25 graft recipients (Table 1). Mean tacrolimus trough level in the CYP3A5*1/*1 group was 5.154 ng/mL (range, 4.42 to 6.5 ng/mL), CYP3A5*1/*3 group was 5.348 ng/mL (range, 3.1 to 9.87 ng/mL) and
The CYP3A5*3/*3 group was 9.483 ng/mL (range, 4.5 to 14.1 ng/mL). Tacrolimus level difference between expressors and nonexpressors was significant when compared with independent $t$ test. ($t$, -4.28; degrees of freedom, 23; $P \leq .001$).

**Effect of CYP3A5 genetic polymorphisms on acute rejection episodes and tacrolimus nephrotoxicity**

Biopsy-proven acute renal graft rejection on biopsies obtained at 1 month after transplant was compared between the 3 CYP3A5 genotype groups. Acute rejection episodes were significantly more frequent for CYP3A5*1/*1 homozygotes (2 out of 5, 40%) than patients with CYP3A5*1/*3 (1 out of 5, 20%) or CYP3A5*3/*3 genotypes (2 out of 15, 13%). We examined the relation between CYP3A5 genetic polymorphism and biopsy-proven nephrotoxicity due to tacrolimus use; 8 renal biopsies were obtained during 1 month, and 2 biopsies (both in nonexpressors) had evidence of calcineurin-induced toxicity.

**Discussion**

Tacrolimus is a potent immunosuppressive drug used in solid-organ transplant. However, it has a narrow therapeutic range, which is further complicated by wide variation in intraindividual and interindividual bioavailability of the drug. Tacrolimus is metabolized by CYP3A4 and CYP3A5 in the liver and small intestine. Genetic polymorphisms in CYP3A5 affect the interindividual variability in tacrolimus trough blood levels.

In our study, we evaluated the effect of CYP3A5 genetic polymorphisms on tacrolimus daily dose requirements in a cohort of kidney transplant recipients. Our results showed that carriers of at least 1 active allele (CYP3A5*1) needed significantly higher doses of tacrolimus than patients homozygous for CYP3A5*3 (CYP3A5 nonexpressors). This result relied on the fact that carriers of CYP3A5*1 allele exhibit high levels of CYP3A5 expression and enzymatic activity, leading to higher daily dose requirement to achieve sufficient trough levels of tacrolimus. Such results have been reported previously in the literature concerning this polymorphism.

A previous study by Patel and associates studied the effect of CYP3A5 polymorphism on tacrolimus drug dosing in North Indian renal allograft recipients. To our knowledge, the present study is the first study to show the association between CYP3A5 genetic polymorphism and tacrolimus drug level in a South Indian population.

We evaluated the risk of biopsy-proven acute rejection during the first month after transplant. We observed that patients with CYP3A5*1/*1 genotype had a higher risk of developing acute graft rejection episodes than CYP3A5*3 homozygotes. This observation is in agreement with the fact that carriers of the wild-type allele (CYP3A5*1) have higher levels of CYP3A5 expression, higher metabolic clearance of tacrolimus, and low trough concentrations resulting in acute rejection.

A previous study by Quteineh and coworkers showed that CYP3A5*1 homozygotes had increased risk of acute rejection episodes (38%) than patients with CYP3A5*1/*3 (10%) or CYP3A5*3/*3 (9%) genotypes ($P = .01$). They also reported that few rejection episodes occurred after the first month after transplant, and overall rejection episodes were more important during the first month after transplant. This showed the importance of performing tacrolimus daily doses early posttransplant, when there is a greater risk of developing acute rejection episodes.

We studied the relation between CYP3A5 genotype and biopsy-proven tacrolimus nephrotoxicity. We observed increased occurrence of nephrotoxicity in CYP3A5 nonexpressors. This was expected because of high trough blood levels in these patients. However, we were limited by the small number of biopsies to substantiate this finding. The previous study by Quteineh and associates showed no relation between the development of tacrolimus-related nephrotoxicity and CYP3A5 genetic polymorphism.

In conclusion, our results confirmed that CYP3A5 genetic polymorphism is an important factor in
determining tacrolimus daily requirements and adjusting tacrolimus trough concentrations. Furthermore, it was shown in our study that genetic polymorphism is a risk factor for developing acute rejection episodes. Screening for this polymorphism in patients waiting for solid-organ transplant could be helpful to predict the best individualized tacrolimus oral dose and may prevent early acute rejection related to insufficient immunosuppression.

References