A STUDY OF POLYMORPHISM OF CYP3A5 GENE AND IT'S EFFECT ON TACROLIMUS BLOOD LEVEL

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Abstract

Tacrolimus is the corner stone of immunosuppression in renal transplantation.It is metabolised byCYP3A subfamily of enzyme in liver and small intestine .A polymorphism in intron 3 of the CYP3A5gene was found to influence the expression of this enzyme and thus tacrolimus trough blood levels.

Objectives

Objectives of our study were to identify the proportion of CYP3A5 gene polymorphism in South Indian renal transplant patients and to determine the impact of CYP3A5 gene polymorphisms on Tacrolimus trough blood levels in CYP3A5 expressors and non expressors.

Materials and Methods

25 adult patients who underwent renal transplantation in Government Medical College, Trivandrum were included in the study. All of them received tacrolimus at a dose of 0.1 mg/kg/body weight.Tacrolimus trough blood levels were determined on sixth post operative day.CYP3A5 genotype analysis was done by Polymerase chain reaction amplification of target followed by detection by Restriction fragment length polymorphism analysis.

Results

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, respectively. Mean Tacrolimus level in CYP3A5 *1/*1 group was 5.154 ng/mL (4.42-6.5) , CYP3A5 *1/*3 group was5.348 ng/mL (3.1-9.87) and CYP3A5*3/*3 group was 9.483 ng/mL(4.5- 14.1). Acute rejection episodes were significantly higher for CYP3A5*1 homozygotes compared to patients with CYP3A5*1/*3 and CYP3A5*3/*3 genotypes (40% % versus 20 % and 13 %, respectively).

Conclusion

Majority of the study population were were carrying the mutant allele CYP3A5*3(A6986G).Tacrolimus drug level correlated well with presence or absence of CYP3A5 Polymorphism.Acute Rejection episodes were more in expressors ,hence they may require more than conventional dose of tacrolimus.Similarly,Tacrolimus nephrotoxicity was more in non expressors.Hence CYP3A5 polymorphism analysis prior to renal transplantation helps in deciding the optimal dose of tacrolimus in this population and helps to prevent both acute rejection episodes as well as Tacrolimus toxicity.

Introduction

Tacrolimus is the corner stoneof immunosuppression in renal transplantation. It is a macrolide antibiotic compound that acts by inhibiting the calcineurin pathway by binding to FK binding protein[1].But it has got a narrow therapeutic index and hence require therapeutic drug monitoring to prevent graft rejection as a result of inadequate immunosuppression and toxicity due to high drug levels.

Tacrolimus is metabolised by CYP3A subfamily of enzymes in liver and in small intestine.Eventhough bothCYP3A4 and CYP3A5 are involved in the metabolism of tacrolimus previous studies have shown that polymorphisms in CYP3A5 genes are responsible for inter individual variations in bioavailability of tacrolimus.[2]

A polymorphism in intron 3 of the CYP3A5gene was found to influence the expression of this enzyme.CYP3A5*3 allele (G at position 6986) produces a cryptic splice site and encodes an abnormal spliced mRNA with a premature stop codon.Hence those individuals who are homozygous for this allele (CYP3A5*3/*3) are called as Non Expressors.Presence of CYP3A5*1allele (A at position 6989) produces a normal mRNA, resulting in a high expression of this enzyme in the intestine and in the liver. Individuals expressing at leastone CYP3A5 *1 allele are called as Expressors .Hence expressors can be either homozygous (CYP3A5*1/*1) orheterozygous (CYP3A5*1/*3). [3, 4]

Previous studies showed that Expressors achieved 2-fold lower Tacrolimus concentrations/ dose ratio compared to Non expressors .[5-9]Hence, 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.

Patients and Methods

25 adult patients who underwent renal transplantation in Government Medical College, Trivandrum, Kerala, India were included in the study.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 of them were receiving Tacrolimus at a dose of 0.1 mg/kg bodyweight along with prednisolone and mycophenolate mofetil.

Tacrolimus12 hour trough blood level (C0) wasdetermined on 6^{th} post-operative day using chromatographic method using LCMS-MS assay. Lower limit of quantification of assay was 0.003ng/mL.

Genotype analyses of all these patients were carried out to identify the CYP3A5 allele. Nucleic acid isolation was done using standard magnetic bead based extraction protocol outlined by the respective kit manufacturers. Custom designed primers synthesized from Homo sapiens cytochrome P450 PCN3 mRNA; complete cds were used for PCR amplification of the target. The PCR protocol followed were a standardized procedure for the selected primer sequence. Post-amplification detection done by Restriction Fragment Length Polymorphism (RFLP) analysis.

Statistical Analysis

Data is described as Mean +/- SD for all quantitative estimates.Tacrolimus trough blood levels between two groups were compared by means of independent t-test .Statistical analysis was done using SPSS software version 16.

Results

Characteristics of study population

Of the 25 renal graft recipients who were included in the study there were 22 men and 3 women. The mean age was 32 ± 20 years and body weight was 58 ± 11 kg.3 patients received Induction therapy, 2 of them received ATG and one received Basiliximab.Mean Donor Age was 44±12 years .At 1-month post-transplantation, the incidence of Biopsy proven Acute Rejection (BPAR) was 20% and allof them were T cell mediated rejection.8 biopsies were obtained from study population in first month 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, respectively. Comparison of the clinical characteristics of the study population between the three CYP3A5genotype groups is given in Table1.Mean Tacrolimus level in CYP3A5 *1/*1 group was 5.154 ng/mL (4.42-6.5) , CYP3A5 *1/*3 group was5.348 ng/mL (3.1-9.87) and CYP3A5*3/*3 group was 9.483 ng/mL(4.5- 14.1). Tacrolimus level difference between expressors and non expressors were significant when compared by using independent t test. (t-4.28 (df=23) $p<0.001$).

Effect of CYP3A5genetic polymorphisms on acute rejection episodes and tacrolimus nephrotoxicity

Biopsy proven acute renal graft rejection obtained at one month post transplantation was compared between the three CYP3A5 genotype groups. Acute rejection episodes were significantly higher for CYP3A5*1 homozygotes compared to patients with CYP3A5*1/*3 and CYP3A5*3/*3 genotypes (40 % versus 20% and 13 % respectively).

We also examined the relation between CYP3A5 genetic polymorphism and biopsy proven nephrotoxicity due to tacrolimus use. Eight renal biopsies were obtained during one month of which 2 had evidence of CNI toxicity .Both of them were non expressors.

Discussion

Tacrolimus is a potent immunosuppressive drug used in solid organ transplantation.[1]But it has got a narrow therapeutic range which is further complicated by wide variation in intraindividual and interindividual variability in bioavailability of drug.Tacrolimus is metabolised by CYP3A4 and CYP3A5 in liver and small intestine. Geneticpolymorphisms in CYP3A5 are found to influence the interindividual variabilityin tacrolimus trough blood levels.[2]

In our study, we evaluated the effect of CYP3A5genetic polymorphismson tacrolimusdaily dose requirements in acohort of kidney transplant recipients. Our results show thatcarriers of at least one active allele (CYP3A5*1) needed significantly higher doses of tacrolimus compared to patientshomozygous for CYP3A5*3 (CYP3A5 non-expressors).This result relies on the factthat carriers of CYP3A5*1allele exhibit high levels ofCYP3A5 expression and enzymatic activity, leadingto higher daily dose requirement to achieve normal trough levels of tacrolimus. Such results have been previously replicated in the literature concerning this polymorphism. [(5-10)]

Previous study by Mohan Patel et al studied the influence of CYP3A5 polymorphism on tacrolimus drug dosing in North Indian renal allograft recipients.[11]To our knowledge, this is the first study to show the association between CYP3A5 genetic polymorphism and Tacrolimus drug level in South Indian population.

We also evaluated the risk of biopsy provenacute rejection during the first month post transplantation. We found that patients with CYP3A5*1/*1genotype hadhigher risk of developing acute graft rejection episodes compared to CYP3A5*3homozygotes. 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, low trough concentrations and, therefore, acute rejection.

Previous study by Lina Quteineh et al had shown that CYP3A5*1homozygotes were associated with increased risk of acute rejection episodescompared to patients with CYP3A5*1/*3and CYP3A5*3/*3genotypes (38% versus 10% and 9%, respectively, P = 0.01).They had also pointed that few rejection episodes occurredafter the first month of transplantation, so the overall rejectionepisodes were more crucial during the first month aftertransplantation. This shows the importance of adapting tacrolimus daily doses in the early period post transplantation, where there is a greater risk of developing acute rejection episodes.[12]

We also studied the relation between CYP3A5 genotype and biopsy proven tacrolimus nephrotoxicity.We found increased occurrence of nephrotoxicity in CYP3A5 non expressors.This was expected in view of high trough blood levels in these patients. But we were limited by small number of biopsies to substantiate this. Butprevious study by Line Quteineh et al found no relation regarding the developmentof tacrolimus related nephrotoxicity and CYP3A5geneticpolymorphism.[12]

In conclusion, our results confirm that CYP3A5 genetic polymorphism is an important factor in determining tacrolimus daily requirements and in adjusting tacrolimustrough concentrations. It was shown, in our study, to beimplicated in the risk of developing acute rejection episodes. Screening for this polymorphism in patients waiting for

solid organ transplantation, could be helpfulto predict the best individualized tacrolimus oral doseand, consequently, prevent early acute rejection related to under immunosuppression.

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Table 1.Comparison of the clinical characteristics of the study population

	CYP *1 / *1 Mean(Range)	**CYP *1/ *3 Mean(Range)**	**CYP *3/ *3 Mean(Range)**
Age at transplantation	16 (12-22)	33 (22-47)	36 (20 – 50)
Sex(M/F)	4/1	5/0	13/2
Age of Donor	44(32-52)	46(37-52)	43(33-58)
Hemoglobin	10.2(8.8-11.8)	10.0(8.4-11.0)	9.8(8.6-10.8)
Blood Urea Nitrogen	18(16-26)	20(15-28)	21(16-27)
S.Creatinine	1.6(0.9-1.8)	1.5(1.0-1.7)	1.6(0.9- 2.4)
S.Potassium	4.2(4.0- 4.4)	4.4(4.0-5.6)	4.4(3.8-5.6)
S.Total bilirubin	1.0(0.8-1.1)	0.8(0.6-1.0)	0.9(0.6-1.1)
ALT	32(26-38)	34(26-36)	36(26-40)
AST	28(26-30)	27(24-32)	30(25-38)
S.Albumin	3.5(3.0-3.8)	3.6(2.8-4.0)	3.5(2.8-4.2)