INTER-INDIVIDUAL VARIATIONS IN TACROLIMUS TREATED PATIENTS WITH RENAL TRANSPLANTS: CASE FOR PHARMACOGENETIC MONITORING

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ABSTRACT

Objectives:Tacrolimus is a widely used drug for renal transplant now a days. In India, it popularity has grown in recent times. One of the important feature of tacrolimus metabolism is that it has wide variation in pharmacokinetics. Main reason of pharmacokinetic variations are variations in intestinal and hepatic metabolism. As a result; there are wide intra and inter-patient variations. However, the incidence of these variations is not clearly known. Pharmacogenetic monitoring of drugs with unpredictable pharmakinetics could be a new tool towards individualization of drug therapy. It has been known for some time that inter-individual variations contribute to drug toxicity and inferior transplant outcomes. We were interested in studying the relationship between dose and concentration in Indian patients who underwent renal transplant and to know its implications. Methods:A total of 102 patients (age range; 17-60 M:F: 87:15; mean age=29.68±20) were prescribed tacrolimus and their blood levels were analyzed to know variations in their blood levels. Oral tacrolimus therapy was initiated at a dose between 0.1 and 0.15 mg/kg/day. For monitoring tacrolimus blood concentration, blood samples were collected between 7 AM and 8 AM. Estimation of tacrolimus blood levels were done using Microparticle Enzyme Immunoassay (i Max-Abbott, USA). The analytic sensitivity of the test is 1.5ng/ml. Results:A total of 102 patients had a mean age of 29.68±20 years (age range 17-65 years). Plasma levels (ng/ml) were 10.82±7.3, 10.6±5.7, 8.92±3.85, 7.89±2.9 in the 1st, 2nd, 3rd and 4th weeks respectively. The respective blood levels mentioned above were obtained with mean dosages (in mg) 7.5±4.6, 7.23±8.1, 5.94±2.5, 5.82±2.7. Multivariate analysis showed no reliable relationship between dose and concentration. Conclusions:Dose response relationship of tacrolimus is poor and routine pharmacokinetic monitoring is needed to identify patients who have low levels of this drug.

Keywords: Tacrolimus, ultra-rapid metabolism, pharmacokinetics, pharmacogenetics

INTRODUCTION

Tacrolimus is a popular immunosuppressive drug following organ transplant. It gained FDA approval for use in liver transplantation in 1994 and, approximately 3 years later, was approved for the prevention of acute rejection in kidney transplantation. Over the last decade or so tacrolimus has become a commonly used the calcineurin inhibitor for the prevention of rejection in renal transplantation [1].

The population pharmacokinetics of tacrolimus in adult kidney transplant recipients shows variability. Thus it is not possible to use standard tacrolimus dosages as an empiric predictor of concentration in this population. An understanding of factors that influence the pharmacokinetics of tacrolimus may assist in drug dosage decisions [2]. That has leaded us to investigate the inter-individual variations in blood tacrolimus levels.

Metabolic enzymes and intestinal transportor polymorphism are the main cause of inter-individual variations. The main impact of the genetic polymorphisms of the metabolic enzymes, efflux transporters is on the dose-concentration and concentration-effect relationships of these drugs. The polymorphisms of metabolic enzymes have significant effects on the pharmacokinetics of tacrolimus, but the clinical trials for validating treatment individualization based on these genetic differences are still lacking.

The influence of efflux transporter genes polymorphisms, in particular of P-glycoprotein and MPR2, is controversial. As for the polymorphisms of the drug targets genes, either they have not been reported (calcineurin, mTOR), or their influence has only been the subject of a few preliminary studies. The pharmacogenetics of immunosuppressants is thus still an open field for investigations and potential therapeutic progress [3].

Variable pharmacokinetics have important implications for both patients and physicians. This is because the tacrolimus blood levels are the only clinically usable indicator of therapeutic response. Due to unpredictable pharmacokinetics; levels may not give a reliable indication of the therapeutic response. The general response of physicians in case of low blood levels is to increase the dose, and in case of high dose is to reduce the drug dose. This may not always be helpful due to intestinal and hepatic factors affecting metabolism. It has been felt that more studies should be conducted to elucidate further the pharmacogenetics of immunosuppressive drugs in organ transplantation, a deep understanding of which would provide an important step toward drug regimen individualization in the posttransplant therapy [4].

Therapeutic drug monitoring remains the most widely used method for individualizing immunotherapy. Improvements in molecular technology have enabled identification of several polymorphisms in genes that encode for drug-metabolizing enzymes, drug transporters, and their targets, which have an effect on individual response to therapy. Prospective studies are needed to explore this field and improve utility of donor and recipient genotype testing in managing immunosuppression therapy [5].

It has been seen that large differences in normal human subjects in the efficacy and safety of many therapeutic agents are caused by genetically controlled polymorphism of drug metabolizing enzymes, drug transporters and drug receptors. Development of pharmacogenomics as a new field has accelerated progress in pharmacogenetics by elucidating at the level of human genome, the inherited basis of those large inter-individual variations [6]. We wanted to know the relationship between dose and response of patients treated with tacrolimus.

MATERIALS AND METHODS

We studied a total of 102 patients (age range; 17-60 M:F: 87:15; mean age=29.68±20). Tacrolimus was given to patients who underwent renal transplantation in an open label manner. Patients were studied and their blood levels scrutinized to know the variations.

Oral tacrolimus (Prograf capsules, 1 mg, 2 mg and 5 mg, Janssen-Cilag, Sydney, NSW, Australia) therapy was initiated at a dose between 0.1 and 0.15 mg/kg/d (in 2 divided doses), 24 hours before transplant surgery.
Tacrolimus dose was then adjusted to maintain blood trough concentrations within the target range (10 to 15 ng/mL) based on the clinical status of the patient. The average daily dose of tacrolimus ranged from 1 to 24 mg/d over the posttransplantation period.

For the monitoring tacrolimus blood concentration, blood samples were collected between 7 AM and 8 AM. Blood samples were collected in Beckton Dickinson Vacutainer tubes containing EDTA and analyzed by the Metropolis Lab* Noida (Utter Pradesh, India), using the Tacrolimus microparticulate enzyme immunoassay (MEIA) method run on the IMx, analyzer (Abbott Laboratories, Abbott Park, IL). The coefficient of variation of the assay (CV) was 8.4% and 14.5% at concentrations of 1.9 and 29.5 ng/mL, respectively, with a working concentration range between 1.5 ng/mL and 30 ng/mL. Biochemical and hematological indices were monitored every day during the patients’ hospital stay.

Estimation of tacrolimus blood levels were done using Microparticule Enzyme Immunoassay (i Max-Abbott, USA). The analytic sensitivity of the test is 1.5 ng/mL.

RESULTS
A total of 102 patients had a mean age of 29.68±20 years (age range 17-65 years). Plasma levels (ng/mL) of these patients were 10.82±7.3, 10.6±5.7, 8.92±3.85, 7.98±2.5 in the 1st, 2nd, 4th and 5th weeks respectively. The respective blood levels mentioned above were obtained with mean dosages (in mg) 7.15±6.1, 7.23±8.1, 5.94±2.5, 5.82±2.7. Multivariate analysis was performed to establish the relationship between the dose of the drug and the plasma level obtained. Linearly independent pair wise comparison among estimated marginal means showed no reliable relationship between dose and concentration.

DISCUSSION
Tacrolimus is the most commonly used immunosuppressive drug in post transplant patients but has variable pharmacokinetics. The drug acts by inhibiting T-cell activation by inhibiting release of cytokines. Tacrolimus was withdrawn from the market after symptoms in 1984 from Japan. The drug binds to an intracellular protein named FKBP-12 to inhibit CD4 activation. Importantly, the drug does not require calcineurin for its activity. However, as a potent immunosuppressive agent, it can cause serious adverse effects, some of which are irreversible and potentially life threatening [7].

Pharmacokinetically, tacrolimus is rapidly absorbed, giving a peak concentration within 0.5 to 1 hour. In whole blood tacrolimus is mainly associated with erythrocytes (80%-95%), and in plasma it predominantly binds to soluble proteins such as albumin and α1-acid glycoprotein, and to a lesser extent to lipoproteins. It is extensively metabolized in the intestinal mucosa and liver mainly by enzymes belonging to CYP3A4 system. Tacrolimus has an apparent clearance of around 20-30 L/h, and several studies suggest that tacrolimus clearance is decreased in patients with hepatic dysfunction.

Present study is an attempt to study the blood levels of tacrolimus in the given population of 102 patients. Four different dosages at weekly intervals were taken and correlated with weekly blood levels of the drug. It appears that there is no dose response relationship between the dose and concentration of drug. It strengthens the notion that the pharmacokinetics of drug is unpredictable and it poses a clinical challenge to settle the appropriate blood levels. It could thus be assumed that we need to know the reasons behind the unpredictability in a given population. That may help us in formulating a better, a tailor made dosage plans for the given patient. Once we know the reasons to be hepatic; then rapid metabolisers could perhaps be offered hepatic microsomal enzyme inhibitors and those with intestinal rapid metabolism could be treated with drugs that can paralyze the intestinal P-glycoproteins. This will help in individualizing the drug treatment and improve tolerability, efficacy and safety. Cost of drug treatment could also be reduced.

The hepatic metabolism of several drugs by cytochrome P450 CYP3A subfamilies is considered a major eliminating process. The intestinal efflux-pump P-glycoprotein ( multidrug resistance 1 [MDR1], ATP-binding cassette B1 [ABC1]) and CYP3A4 have been demonstrated as important for the bioavailability of drugs, so called "absorptive barriers". Recently, an important role for CYP3A5 in the intestine for the oral clearance of drugs has been identified. Both tacrolimus and cyclosporine are substrates of P-glycoprotein, CYP3A4 and CYP3A5, and therefore, these molecules are potential pharmacokinetic factors with which to establish personalized dosage regimens for these drugs. Although the effect of single nucleotide polymorphisms in the MDR1/ABC1 and CYP3A5 genes on the pharmacokinetics of immunosuppressant has been widely examined, some contradictions have been emerged. In living donor liver transplant (LDLT) patients, the intestinal mRNA expression level of MDR1 and CYP3A5 genotyping both in the native intestine and in the grafted liver are suggested to be potential pharmacokinetic factors for adjusting initial dosage and predicting post-operative variation in the pharmacokinetics of tacrolimus. Implications of this study is the need to establish the existence of inter-individual variations and know its relevance in Indian context; this will serve as a means of knowing the impact of inter-individual variations on the drug treatment and devising the way in which patient behave on tacrolimus.

The variability in tacrolimus metabolism has been attributed to interindividual differences in the expression of the metabolizing enzymes cytochrome P450 (CYP) 3A4 and 3A5, and in the expression of the drug transporter P-glycoprotein (encoded by the ABCB1 gene, formerly known as the multidrug resistance 1 gene). Variation in the expression of these genes could in turn be explained by several recently-identified single nucleotide polymorphisms. Determination of these SNPs in prospective patients has the potential to identify individuals who are at risk of under-immunosuppression or the development of adverse drug reactions. Ultimately, genotyping for CYP3A and ABCB1 may lead to further individualization of immunosuppressive drug therapy for the transplanted patient. For this reason, apart from pharmacokinetic monitoring; of calcineurine inhibitors has been suggested. However, considering many polymorphism types affect the metabolism of tacrolimus; finding out the microsomal enzyme/intestinal polymorphism type may not be easy to find. In a recent study from India, tacrolimus drug level correlated well with presence or absence of CYP3A5 polymorphisms. It has been realized that the pharmacokinetics of immunosuppressives like tacrolimus greatly affect their in vivo performance. In spite of its success in ensuring graft survival, therapeutic use of tacrolimus is complicated due to its narrow therapeutic index. It has already been stated that tacrolimus has a large inter-/intra-patient variability in pharmacokinetics profile and a poor oral bioavailability because of its poor solubility, P-glycoprotein efflux, marked pre-systemic metabolism by CYP3A in the enterocytes and liver first pass effect; this strengthens the case for its pharmacogenetic monitoring in Indian subjects.

CONCLUSIONS
Pharmacokinetics of tacrolimus is unpredictable and there is no reliable dose and concentration relationship. Inter-individual variations exist in a sizable number of patients and this can have an important bearing in using tacrolimus based regimen. Increase in risk of side effects, suboptimal efficacy, transplant rejections are some of the fallouts of inter-individual variations. Currently, the only reliable means of individualizing drug therapy is plasma level estimation. Identification of gene/s contributing metabolic variations and establishing them at population levels in India is suggested.

There is a need to explore more on this issue as to know, what could be the possible reason of variations, what are the implications, how could we establish dose and response relationship and what recommendations can be made regarding the treatment of patients with tacrolimus based regimen. Exploring the role of genetic polymorphism and knowing the contribution of intestinal transporters in bioavailability of tacrolimus is an upcoming area of research.

REFERENCES