Tacrolimus Therapeutic Drug Monitoring in Stable Kidney Transplantation and Individuation of *CYP3A5* Genotype

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ABSTRACT

The posology of tacrolimus (TAC) is usually guided by its therapeutic drug monitoring. Some patients reach target concentrations (CTs) quickly, others more slowly. In a retrospective study, 20 kidney transplant recipients were included (mean age, 50.7 ± 14.1 years; weight 64.0 ± 14.2 kg; patients clinically stable for over a year). We studied cytochrome *CYP3A5* genotype, in particular *CYP3A5* 6986A>G, the most important polymorphism related to the metabolism of TAC (wild genotype CYP3A5 *1 genotype, and CYP3A5 *3 variants). One year after transplantation, the CTs were 5.0 to 8.0 ng/mL. The patients were divided into group A (TAC doses < 6.0 mg/d) and group B (TAC doses > 6.0 mg/d). All were tested for the *CYP3A5* gene sequence to characterize their polymorphism. Patients with CYP3A5 *1/*1 and *1/*3 were extensive metabolizers, and those with CYP3A5 *3/*3 were poor metabolizers. In group A and group B, the average TAC doses at the time of therapeutic drug monitoring were 3.0 ± 1.4 ng/mL (0.05 ± 0.03 mg/kg) and 12.8 ± 3.7 ng/mL (0.2 ± 0.1 mg/kg), respectively ($P < .001$). Group A was the poor metabolizers genotype, while in group B, the extensive metabolizers genotype was present. Patients with the CYP3A5 *1/*1 or *1/*3 genotype required 1.5 to 2 times higher doses than patients *3/*3 to reach CT. This genetic test allows clinicians to know, before the kidney transplant, the patient's TAC metabolism pattern and then to optimize the drug exposure.

TACROLIMUS (TAC), a calcineurin inhibitor, is an immunosuppressive agent used in the prevention of graft rejection in kidney transplant recipients [1-3]. It was first introduced for clinical use in transplantations in 1989 and is used as part of the immunosuppressant regimen for more than 90% of all kidney transplant recipients [4]. The clinical use of TAC is complicated by its high pharmacokinetic variability among patients as well as its narrow therapeutic index [5].

A patient's initial TAC dose is conventionally determined based on body weight and is adjusted according to therapeutic drug monitoring (TDM) of trough concentrations (C_0). Because of its narrow therapeutic index, TDM is used regularly with the goal of optimizing the fine balance between graft rejection and drug toxicity [6]. The C_0 of TAC in kidney transplant recipients varies according to days post-transplantation, concomitant drug therapies, aspartate aminotransferase, alanine aminotransferase, and serum creatinine. Each post-transplant period has its own target concentration (CT), but patients do not always reach it. Patients, who do not reach the CT could have a graft-rejection risk increase because of underexposure of TAC, while the patients who exceed the CT could have clinical toxicity.

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Drug-metabolizing enzymes in the CYP3A subfamily also heavily influence TAC pharmacokinetics. The presence of *CYP3A4* and *CYP3A5* in the intestinal mucosa and in the hepatic cells contributes to a first-pass effect as the drug molecules are metabolized prior to reaching the systemic circulation. Genetic polymorphism in these 2 enzymes account for a significant part of the inter-individual variability observed in the TAC bioavailability. The best studied genetic variation is in the *CYP3A5* gene [7]. *CYP3A5* plays a dominant role in the metabolism of TAC, and polymorphism of its gene could alter TAC metabolism [8]. Up to 9 different alleles have been identified in the *CYP3A5*. The best-studied polymorphism is the single-nucleotide polymorphism 6986A>G (rs776746). The mutant variant bearing the G nucleotide is denoted by *3 alleles. In most ethnic populations, except black people, homozygous *3/*3 is the most frequent genotype and is associated with *CYP3A5* non-expressers. These patients need a normal daily dose of TAC because they have whole blood concentrations that are normal or higher. The *CYP3A5* wild-type variant bearing A nucleotide (*1/*1 allele) and the heterozygous AG variant (*1/*3 allele) are associated with the *CYP3A5* enzyme expresser. These patients need a higher daily dose of TAC because they have whole blood concentrations that are lower [7]. The *3/*3 alleles found in ethnic populations is present in 82% to 86% of white people, but 13% to 17% of white people have the *1/*3 or *1/*1 alleles; African people mainly have the *1/*3 and *1/*1 alleles (40%-54% and 37%-45%, respectively). Indian and Chinese patients are prevalently *1/*1 (32.6% and 47.4%, respectively) and *1/*3 (38%-57%, and 44.8%, respectively). Guidelines on the correct use of TAC TDM have been published based on the genetic analysis of *CYP3A5* [9].

However, this is advice for clinicians to optimize therapy with TAC. For this reason, we wanted to analyze a small number of kidney-transplanted patients with the same clinical characteristics who presented significant disparities in TAC daily doses. We studied the *CYP3A5* genotype as part of a routine TDM of TAC, particularly focusing on the polymorphism *CYP3A5* 6986A>G.

MATERIALS AND METHODS

Patients and Data Collection

This is a retrospective study of 20 adult patients who underwent kidney transplantation in 2016 at the Complex Operating Structure (SOC) Nephrology, Dialysis, and Kidney Transplantation unit in the Integrated University Hospital of Udine, Italy, and who had TAC TDM performed by the SOC Institute of Clinical Pharmacology of the same hospital. Transplanted kidney patients, who had stable hepatic and renal function, were treated with TAC, mycophenolate mofetil, and prednisone. The patients were not taking drugs inducing or inhibiting the TAC. One year after transplantation, TAC doses were then adjusted to achieve a trough CT between 5.0 and 8.0 ng/mL.

TAC whole-blood concentrations were determined by the immunoassay method automated antibody conjugated magnetic immunoassay on a Siemens Dimension Integrated Chemistry Systems Tacrolimus (Siemens Healthineers, Erlangen, Germany). The mean intraday precision values ± standard deviation for low, medium, and high TAC were 4.36 ± 0.26 ng/mL, 9.14 ± 0.33 ng/mL, and 17.34 ± 0.71 ng/mL, respectively. The mean inter-day precision values ± standard deviation for low, medium, and high TAC were 4.28 ± 0.24 ng/mL, 9.18 ± 0.26 ng/mL, and 17.16 ± 0.67 ng/mL, respectively. The analyses of the TAC TDM were performed daily as routine analyses.

Genotyping of *CYP3A5* Polymorphism

After a signed informed consent, blood samples of proband and parents was obtained. For *CYP3A5* genotyping, genomic DNA was isolated from an EDTA peripheral blood sample using QIAamp Blood Midi Kit according to the manufacturer procedure (Qiagen, Hilden, Germany). In order to characterize patients' *CYP3A5* genotype, screening for *CYP3A5* *1/*1, *1/*3, and *3/*3 polymorphisms were performed by Sanger sequencing. Sequencing reactions were analyzed by a 3500 DX DNA Sequencer (Life Technologies, Carlsbad, CA, United States) analyzer using the BigDye Terminator kit v3.1 (Applied Biosystems, Foster City, CA, United States); the software Sequencing Analysis v5.4 (Life Technologies) was used for mutation detection. Patients with *CYP3A5* *1/*1 and *1/*3 were considered extensive metabolizers (EMs), and ones with *CYP3A5* *3/*3 were poor metabolizers (PMs) [9]. The genetic analyses were performed by the guidelines of the SOC Institute of Medical Genetics of the Integrated University Hospital of Udine, Italy. The study was approved by the Internal Review Board of Udine University.

Data Processing

After performing the pharmacogenetics analyses on all patients, the group was divided into 2 parts based on the TAC mean daily doses: group A patients had TAC daily doses < 6.0 mg/24 hours; group B patients had TAC daily doses > 6.0 mg/24 hours. To get a normalization, the doses were transformed into 1.0 mg/kg. For statistics we have used Sigma Stat (SigmaPlot 2004 for Windows Version 9.0, Systat Software, Inc, San Jose, CA, United States), and the results were considered significant when $P < .05$.

RESULTS

The demographics, biological, and pharmacogenetic characteristics of the 20 patients in this study are summarized in Table 1. Their mean age was 50.7 ± 14.1 years, and the mean weight was 64.0 ± 14.2 kg. The clinical outcomes were 1 patient with acute rejection and subsequent death and 1 patient with chronic rejection. All patients had reached CTs, and the mean daily dose of TAC at 1 year was 7.9 ± 5.7 mg (0.13 ± 0.09 mg/kg). In all 20 renal transplant recipients, the frequency distribution of *CYP3A5* *1/*1, *1/*3, and *3/*3 genotypes were 1 (5%), 5 (25%), and 14 (70%), respectively (Table 2). The PMs presented a mean dose of 3.0 ± 1.4 (0.05 ± 0.03 mg/kg), while EMs required a higher dose of TAC to reach the CT, which was 12.8 ± 3.7 mg (0.2 ± 0.1 mg/kg, $P < .001$). From ethnic pharmacogenetics point of view, group A were predominantly 10 white people with *CYP3A5* *3/*3, while group B had 4 white people with *CYP3A5* *3/*3; 3 white people, 1 African, and 1 Asian with *CYP3A5* *1/*3; and 1 African with *CYP3A5* *1/*1.

Table 1. The Demographics and Biological Characteristics of the 20 Patients Studied

Characteristics	All the Patients
Patients, n°	20
Sex, M/F	11/9
Age, y	50.7 ± 14.1
Weight, kg	64.0 ± 14.2
Height, cm	167.6 ± 12.6
BMI, M/F, kg/m^2	23.21 ± 3.69
	22.4/5.04
Cr, mg/dL	1.4 ± 0.4
ClCr, mL/min	59.1 ± 19.6
AST T_{12}, U/L	15.4 ± 2.5
ALT T_{12}, U/L	15.1 ± 8.8
TAC daily dose, mg	7.9 ± 5.7
TAC dose/kg, mg/kg	0.13 ± 0.09
Prednisone, mg/24 h	3.8 ± 0.7
MMF, mg/24 h	1039.4 ± 597
Acute rejection	1
Chronic rejection	1
Death	2

Values are expressed as median ± SD.
Abbreviations: Alb, serum albumin; ALT, alanine aminotransaminase; AST, aspartate aminotransferase; BMI, body max index; ClCr, clearance creatinine; Cr, plasma creatinine; Htc, hematocrit; M/F, male-to-female ratio; MMF, mycophenolic acid; TAC, tacrolimus; T_{12}, 12 months after kidney transplantation.

DISCUSSION

TAC is one of the most commonly used immunosuppressant and has been extended as a first-line regimen for kidney, heart, lung, intestinal, and bone marrow transplantation.

In an attempt to minimize and even avoid organ rejection and adverse drug reactions, it is essential to achieve desired target blood drug concentrations shortly after transplantation [5,10]. To do that, daily dose adjustments would be made based on whole-drug concentrations at a steady state after determining the optimal TAC dosage within the first several days post transplantation [2]. Inter-individual variability in the pharmacokinetics of TAC should be taken into account among patients as well as its narrow therapeutic index. This can lead to underexposure, which may potentially increase the risk of rejection, or to overexposure, which may potentially increase the risk of toxicity such as nephrotoxicity, delayed graft function, posttransplant diabetes mellitus, hypertension, and neurotoxicity [11]. Unfortunately, the pharmacodynamics, pharmacokinetics, and narrow therapeutic index of TAC vary dramatically among individuals. The variability of TAC concentrations depends partly on changes in the *CYP3A5* gene. In kidney transplants, individuals with *CYP3A5* *1/*1 or *1/*3 genotypes may require TAC doses 1.5 to 2 times the dose required to reach the CT [9]. The results of this study confirmed this observation.

The majority of patients required a standard dose (3.0 mg) of TAC and were in group A (PM) with *CYP3A5* *3/*3 (100%) characteristics. The patients in group B, which required a higher dose of TAC (13.0 mg), presented with different genetic characteristics: *CYP3A5* *3/*3 (40%), *1/*1 (10%), and *1/*3 (50%). While the patients in group A were all white, the patients in group B were from different ethnic groups: 7 patients were white, 2 Africans, and 1 Asian. Therefore, even in white patients there could be people who require higher TAC doses. These patients were all EMs, 4 with *CYP3A5* *3/*3 and 3 with *CYP3A5* *1/*3, probably because the only single-nucleotide polymorphism 6986A>G (rs776746) was studied. Therefore, we recommend studying as many genetic variations as possible.

Table 2. Mean Parameters ± SD of Groups A and B with Statistically Significant Differences

Genotype	Number of Patients; Sex	Body Weight, kg	TAC Dose, mg/d	TAC Dose, mg/kg/d
Group A PM	10; 5/5	57.2 ± 13	2.9 ± 1.4	0.05 ± 0.03
Group B EM	10; 6/4	44.2 ± 12.5*	12.7 ± 3.7†	0.2 ± 0.1†

Values are expressed as median ± SD.
Abbreviations: EM, extensive metabolizers (CYP3A5 *1/*1 and *1/*3); PM, poor metabolizers (CYP3A5 *3/*3); SD, standard deviation; TAC, tacrolimus.
*$P < .05$.
†$P < .001$.

Terrazzino et al [12] published a meta-analysis to estimate the effects of CYP3A5 6986A>G polymorphism on TAC dose-adjusted C_0 in kidney transplants. The goals of genotype-based dosing is to provide empirical dosing that allows for the required achievements of the CT, particularly in the initial days after transplantation. Therefore, patients carrying the *CYP3A5* *3/*3 genotype will require a lower dose of TAC to achieve the same blood concentrations when compared with the *CYP3A5* *1/*1 or *CYP3A5* *1/*3 carriers [12].

In another retrospective study, an empirical weight-base starting dose of 0.1 mg/kg was used to target a therapeutic range of 4.0 to 8.0 ng/mL. Among *CYP3A5* "non-expressers" (*CYP3A5* *3/*3), 50% of patients achieved C_0 within the target range by day 3. Among *CYP3A5* "expressers" (*CYP3A5* *1/*3 or *1/*1), however, only 35.3% of patients achieved C_0 within the therapeutic range by day 3. But after genetic study, 64.2% of expressers achieved the therapeutic drug concentrations. These results suggest that *CYP3A5* genotyping is likely more useful if it would be available before kidney transplantation [13]. African , who more frequently carry a *CYP3A5* *1/*1 allele and have a high risk of poorer outcomes, may benefit most from genotype-directed dosing [14–16].

In a multiethnic population, this variable must also be taken into account in order to reach the CT of TAC as soon as possible after organ transplantation and avoid rejection.

Certainly, this publication has a limited number of patients, and therefore the results obtained could be questionable. However, even in only a year of transplants, differences were found due to *CYP3A5*. To date, the researchers have not shown any benefit in clinically important outcomes, such as acute rejection, nephrotoxicity, or graft survival, with the use of genotype-based assays.

Another problem is the analytical method. It is known that the gold standard is Liquid Chromatography Mass

Spectrometry (LC-MS/MS) [14], but not all laboratories have this equipment. The TAC dosage and CT recommendations for TAC vary from center to center, and large pharmacokinetic variability makes it difficult to predict what concentration will be achieved with a particular dose or dosage change. However, since the target of this study is the genetic aspect of cytochrome-p450, the analysis performed with an immunoassay does not affect the result. In particular, it was shown that the analysis with the automated antibody-conjugated magnetic immunoassay was not affected by *CYP3A5* polymorphisms [17].

In a normal clinical routine, usually the pharmacogenetics analysis is not performed. The results of this study show that it is possible to find EM even on 20 patients. These patients require more days to reach the CT within the first month. This could increase the risk of rejection. The *CYP3A5* genotype could be identified before transplantation in order to achieve the CT earlier.

CONCLUSION

In conclusion, a 1-year retrospective analysis in kidney transplant recipients demonstrated the presence of a genetic variation that leads to a different metabolization of the TAC. Faster achievement of CTs could potentially reduce the risk of graft-rejection due to underexposure. This is a personalization of therapy that improves a transplantation service. This study shows that the cooperation between the nephrology and surgery, clinical pharmacology, and genetics services may optimize therapy with TAC to achieve faster CT in order to reduce the risk of rejection and welfare costs.

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