Published in final edited form as:

Xenobiotica. 2013 July ; 43(7): 641–649. doi:10.3109/00498254.2012.752118.

Concentration of Tacrolimus and Major Metabolites in Kidney Transplant Recipients as a Function of Diabetes Mellitus and Cytochrome P450 3A Gene Polymorphism

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Abstract

1. Disposition of tacrolimus and its major metabolites, 13-O-desmethyl tacrolimus (13-DMT) and 15-O-desmethyl tacrolimus (15-DMT), was evaluated in stable kidney transplant recipients in relation to diabetes mellitus and genetic polymorphism of cytochrome P450 (CYP) 3A.

2. Steady-state concentration-time profiles were obtained for 12-hour or 2-hour post dose, in twenty (11 with diabetes) and thirty-two (24 with diabetes) patients, respectively. In addition, single nucleotide polymorphisms of the following genes: CYP3A4 (*CYP3A4*: *CYP3A4*1B*, -392A>G), 3A5 (*CYP3A5*: *CYP3A5*3*, 6986A>G) and P-glycoprotein (*ABCB1*: 3435C>T), were characterized.

3. Dose-normalized exposure to tacrolimus or metabolites were higher in diabetic patients. *CYP3A4*1B* carriers and *CYP3A5* expressers, independently or when considered as a combined *CYP3A4-3A5* genotype, had significantly lower dose-normalized pre-dose (C_0 /dose) and 2-hour post dose (C_2 /dose) concentrations of tacrolimus and metabolites. Nondiabetic patients with at least one *CYP3A4*1B* and *CYP3A5*I* allele had lower C_0 /dose as compared to the rest of the population.

4. Genetic polymorphism of CYP3A5 or CYP3A4 influence tacrolimus or metabolites dose normalized concentrations but not metabolite to parent concentration ratios. The effect of diabetes on tacrolimus metabolism is subject to debate and requires a larger sample size of genetically stratified subjects.

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Declaration of Interest: The authors have no conflicts of interest to report. The authors alone are responsible for the content and writing of this manuscript.

Keywords

Clearance; pharmacokinetics; CYP3A5*1; CYP3A4*B

Introduction

The calcineurin inhibitor tacrolimus is an essential component of immunosuppressant therapy after organ transplantation (Staatz and Tett, 2004). Tacrolimus exhibits a narrow therapeutic index and variable pharmacokinetic characteristics with oral bioavailability ranging from 4 to 89%. Therefore, routine therapeutic drug monitoring is an integral part of tacrolimus regimen after organ transplantation (Staatz and Tett, 2004; Venkataramanan *et al.*, 1995).

Tacrolimus undergoes extensive oxidative biotransformation that is mediated by hepatic and intestinal cytochrome P450 (CYP) 3As (Lampen *et al.*, 1995; Karanam *et al.*, 1994). More than 90% of the absorbed tacrolimus dose is biotransformed into several metabolites that are excreted via the biliary route (Venkataramanan *et al.*, 1995). These include three mono-demethylated at 13-, 15-, and 31-methoxy group (also known as M-I, M-III and M-II), three didemethylated, at 13- and 15-, 15- and 31-, and 13- and 31-methoxy group (also known as M- VII, M-V and M-VI) and one mono-hydroxylated at the 12-position (also known as M-IV) metabolites (Iwasaki *et al.*, 1993). Among these metabolites, the formation of 13-O-desmethyl tacrolimus (13-DMT) is the primary route of tacrolimus biotransformation (Sattler *et al.*, 1992; Staatz and Tett, 2004; Iwasaki, 2007). Most tacrolimus metabolites, with the exception of M-II are pharmacologically inactive but their toxicological activity is not known (Iwasaki, 2007). Moreover, some metabolites cross-react with immunoassay methods used to measure tacrolimus concentration resulting in the over-estimation of the parent drug concentration (Ansermot *et al.*, 2008).

Although CYP3A4 is the most abundant P450 isoenzyme that is present in human liver and intestine, several studies have shown that CYP3A5 plays a crucial role towards the formation of tacrolimus metabolites (Sattler *et al.*, 1992; Kamdem *et al.*, 2005). Limited literature is available on phase II biotransformation of tacrolimus. In this regard, only one study has reported the presence of glucuronide conjugates of tacrolimus in transplant recipients (Firdaous *et al.*, 1997). In addition, a recent *in vitro* investigation demonstrated that tacrolimus glucuronidation is mainly catalyzed by uridine diphosphate-glucuronosyltransferase (UGT) 1A4 (Laverdiere *et al.*, 2011). Moreover, tacrolimus is a substrate for the efflux transporter, P-glycoprotein (P-gp), an encoded product of *multidrug resistance 1* gene (also known as *ABCB1*) that is a member of ATP-binding cassette transporters (Saeki *et al.*, 1993).

The global prevalence of diabetes is increasing at a rapid rate and it is estimated that the number of people with diabetes will rise from 171 million in 2000 to 366 million in 2030 (Wild *et al.*, 2004). Data from the United Network for Organ Sharing (UNOS) indicates that ~32% of all kidney transplant recipients in the United States are diabetic prior to their transplant operation while another ~10% develop new-onset diabetes after transplantation (NoDAT) (Kuo *et al.*, 2010). Furthermore, chronic hyperglycemia and altered levels of

hormones and cytokines may alter the activity of drug metabolizing enzymes and transporters in patients with diabetes (Cheng *et al.*, 2001). Despite this, expression and activity of major human phase I and II drug metabolizing enzymes, under diabetes condition, was reported only in a few studies (Cheng *et al.*, 2001; Dostalek *et al.*, 2011a; Dostalek *et al.*, 2011b). These alterations along with other factors such as altered drug absorption, protein binding and distribution (Zini *et al.* 1990, Dostalek *et al.* 2012a) may have a considerable impact on the concentration of tacrolimus and its major metabolites.

The CYP3A and P-gp collectively contribute to the reduced bioavailability of tacrolimus. The most common polymorphism of *CYP3A4* gene, *CYP3A4*1B* (-392A>G, rs2740574), is reported to be associated with prostate cancer and leukaemia caused by exposure to epipodophyllotoxins (Lamba *et al.*, 2002). In some *in vitro* studies, transfection of variant *CYP3A4*1B* sequence leads to increased CYP3A4 transcriptional activity (Staatz *et al.*, 2010). *CYP3A5* gene is also expressed in a polymorphic form in the population (Kamdem *et al.*, 2005). Among several genetic variants discovered, *CYP3A5*3* (6986A>G, rs776746) causes an alternative splicing and was associated with low CYP3A5 protein content (Hustert *et al.*, 2001; Kuehl *et al.*, 2001). Presence of at least one *CYP3A5*1* allele was associated with reduced oral bioavailability of CYP3A5 substrates (Staatz *et al.*, 2010) and increased *in vitro* biotransformation in the liver (Dai *et al.*, 2006). In addition, 16 different single nucleotide polymorphisms (SNPs) of *ABCB1* gene have been identified. Among them, an SNP in exon 26 (3435C>T, rs1045642) was associated with higher P-gp activity, leading to lower plasma concentration of digoxin and phenytoin (Hoffmeyer *et al.*, 2000; Kerb *et al.*, 2001). However, the effect of *ABCB1* 3435C>T on tacrolimus dose requirement is subject to debate (Staatz *et al.*, 2010).

To date, only two studies have characterized tacrolimus disposition in diabetic patients. In 7 diabetic and 11 nondiabetic patients awaiting kidney transplantation, van Duijnhoven *et al.* (1998) have observed that diabetic patients exhibited ~38% lower median tacrolimus exposure than nondiabetic patients. However, Mendonza *et al.* (2007), in stable kidney allograft recipients, found a trend toward higher values of tacrolimus dose-normalized AUC₀₋₁₂ (area under the concentration-time curve from 0 to 12-hours) and C_{max} (maximum concentration) in the diabetic group. Furthermore, because of the significant role of CYP3A4, 3A5, and P-gp on tacrolimus pharmacokinetics, patients with a functional genetic variant may require a higher tacrolimus dose to achieve similar therapeutic concentrations than patients carrying a non-functional variant. The objective of this study was to evaluate the combined effect of diabetes and gene polymorphism on the dose-normalized concentration of tacrolimus and metabolites.

Methods

The Institutional Review Board of Rhode Island Hospital, Providence RI, USA, approved the study protocol. The protocol was verbally explained and written informed consent was obtained from all the subjects prior to pharmacokinetic sampling.

Study population

Detailed demographic information is presented in Table 1. Study I involved twenty (11 diabetic and 9 nondiabetic) and Study II involved thirty-two (24 diabetic and 8 nondiabetic) adult stable kidney transplant recipients. Diabetic and nondiabetic patients were broadly matched based on their age, time post transplant and concomitant medications and the proportion of African American patients was comparable between the two groups. Patients were excluded if they were suffering from severe liver dysfunction, were pregnant, nursing or younger than 18 years of age. In addition, patients with a pancreas transplant were excluded. All study participants received a triple immunosuppressive drug regimen that included tacrolimus oral tablets (Prograf, Astellas Pharma US, Inc., Deerfield, IL, USA), prednisone and mycophenolic acid either from mycophenolate mofetil (Cellcept™, Roche, Nutley, NJ, USA) or mycophenolate sodium (Myfortic, Novartis, East Hanover, NJ, USA).

Pharmacokinetics study

On the day of pharmacokinetic study, subjects underwent routine physical examination including blood pressure, height and weight measurement. Tacrolimus dosing was at steady-state for all patients and was typically adjusted by the transplant physician based on the results of the routine therapeutic drug monitoring to achieve the trough concentration within the target levels (10-12 ng/mL for the first 6 months and 4-6 ng/mL thereafter). After collecting a pre-dose (trough) blood sample (4.0 mL) into ethylenediaminetetraacetic acid (EDTA) Vacutainers® (Becton Dickinson; Franklin Lakes, NJ, USA), immunosuppressive drugs were administered. Blood samples from Study I subjects (0.25, 0.5, 1, 1.5, 2, 3, 5, 7, 9, 10 and 12 hours post-dose) were collected over a period of 12-hours, whereas only two blood samples (pre-dose and 2-hours post-dose) were collected from Study II subjects. Study I subjects were asked to fast from the night before but were served standardized diabetic meal (comprising a total of 2000 Kcal/day) at around 2, 5 and 10 hours post-dose.

Quantitative analysis of tacrolimus and its metabolites

The whole blood samples were immediately stored at -80°C until further analysis. Quantitative analysis of tacrolimus and its metabolites was performed at iC42 Clinical Research & Development, Department of Anaesthesiology, University of Colorado Denver, Aurora, CO, USA.

A previously published and utilized assay (Christians *et al.*, 2000; Mancinelli *et al.*, 2001) was slightly modified and validated using an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) coupled to an API 4000 tandem mass spectroscopy system (AB Sciex, Foster City, CA, USA) equipped with a turbo electrospray ion source. In brief, sample preparation involved the addition of 800 µL of ZnSO₄ (17.28 g/L): methanol (30:70, v/v) containing the internal standard (ascomycin, 100 ng/mL) to a 200 µL aliquot of EDTA anticoagulated whole blood, calibration standards or quality control samples. Samples were vortex mixed, centrifuged (13,000 rpm for 10 minutes at 4°C), and 50 µL of supernatant was injected onto an HPLC column (4.6 × 150 mm, 3.5µm, Eclipse Zorbax XDB -C8, Agilent Technologies) maintained at 65°C. The mobile phase that consisted of (A) HPLC grade methanol with 0.1% v/v formic acid and (B) 0.1% v/v formic acid was pumped at a flow rate of 1.0 mL/min. The mass spectrometer was run in the single ion mode

and focused on the $[M+Na]^+$ of tacrolimus, ascomycin (internal standard), and the major tacrolimus metabolites. Metabolite peaks were identified in the ion spectra by comparison with the HPLC retention times and mass spectra of authentic standard material (Mancinelli *et al.*, 2001). Calibration curves were constructed using quadratic $1/x$ regression by plotting nominal concentration versus analyte area/internal standard area ratios. The tacrolimus metabolite concentrations were estimated semi-quantitatively using tacrolimus calibration curves (Mancinelli *et al.*, 2001). The lower limit of quantification (LLOQ) for tacrolimus and metabolites in human EDTA whole blood was at 62.5 pg/mL, the range of reliable response was from 62.5 pg/mL to 25.0 ng/mL. Inter-day accuracies for tacrolimus was within 85-115% and total imprecision was <15%. There were no significant matrix interferences, ion suppression/enhancement and carry-over. Tacrolimus and its metabolites were stable in the autosampler at 4°C for 24 hours and EDTA whole blood samples could undergo at least 3 freeze-thaw cycles.

Evaluation of genetic polymorphism

Genomic DNA from peripheral blood sample of each patient was extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions and was stored at -80°C until analysis. *CYP3A4*1B* was genotyped by the use of polymerase chain reaction amplification and direct sequence analysis of portions of the *CYP3A4* gene as described previously (He *et al.*, 2005). SNPs of *CYP3A5* and *ABCB1* gene were determined by TaqMan® allelic discrimination assay (Life Technologies Corporation, Carlsbad, CA, USA) using an Applied Biosystems 7500 Real-Time PCR system according to manufacturer's instructions.

Data analysis

Pharmacokinetic analysis of tacrolimus, 13-DMT and 15-O-desmethyl tacrolimus (15-DMT) was performed using WinNonlin software version 5.0.2 (Pharsight, Mountain View, CA). All the basic pharmacokinetic parameters including, AUC_{0-12} , C_{max} , time to reach maximum concentration (T_{max}) and clearance were calculated for Study I patients using non-compartmental model with extra-vascular input.

All statistical analysis was performed using the SPSS software (version 19.0, IBM SPSS Statistics, Chicago, IL, USA). Gaussian distribution of all the data was verified using the Shapiro-Wilk test and reported as mean $\pm$ SD and significance level was tested using an independent samples t test. The non-normally distributed data are presented as median and inter-quartile range or geometric mean and 95% confidence interval, and were subjected to the Mann-Whitney U test. To compare more than two groups, an Analysis of Variance (ANOVA) was performed in combination with Bonferroni t-test as post-hoc analysis. Because of the small sample size in some groups presented in Figure 2, log transformed values of dose normalized concentrations were subjected to General Linear Model Univariate analysis of variance with pairwise comparisons. The obtained P values between the observed data were then compared with that obtained when 1000 bootstrap samples performed using Wild sampling method.

Results

Tacrolimus morning dose in the combined dataset (study I and II) was 2.48 (2.28, 2.69) mg in diabetic and 3.47 (3.09, 3.90) mg in non-diabetic patients (geometric mean and 95% confidence interval, $P=0.021$). Biochemical indices were similar except significantly higher levels of haemoglobin A1c and blood glucose in diabetic patients (Table 1). None of the study subjects were administered drugs that are known to inhibit or induce tacrolimus disposition.

Effect of diabetes on the disposition of tacrolimus and metabolites

Comparison of tacrolimus pharmacokinetic parameters (Figures 1A, 1B and Table 2) in patients from Study I showed that diabetic patients have significantly longer T_{max} , and lower apparent clearance (Table 2). Figure 1C and 1D illustrate the concentration-time profiles of 13-DMT and 15-DMT, respectively. Dose-normalized AUC_{0-12} and C_{max} of 15-DMT were significantly higher in diabetic patients, whereas, these parameters were only marginally higher for 13-DMT (Table 2).

Since the concentration of 13, 15-O-didesmethyl tacrolimus was below the LLOQ at several time points, we could only analyze its concentration in 42% (100 out of 238) and 67% (42 out of 63) of the samples from Study I and II, respectively. The comparison of pooled concentrations of 13, 15-O-didesmethyl tacrolimus in diabetic and nondiabetic groups from Study I showed significantly higher concentration in the diabetic group (median concentration/dose: diabetic=0.25 ng/mL vs. nondiabetic=0.05 ng/mL, $P<0.001$). A similar trend was observed among Study II patients, although the data did not reach statistical significance. In addition, the concentration of 31-O-desmethyl or 12-hydroxy tacrolimus were below LLOQ in all samples.

Effect of gene polymorphism on tacrolimus and metabolite concentrations

The sample size of 12-hour pharmacokinetic study (Study I) was insufficient to evaluate the combined effect of diabetes and genetic polymorphism on tacrolimus disposition. Thus, to increase the sample size, thirty-two additional patients were recruited and sampled at pre-dose (C_0 /dose) and 2-hour post-dose (C_2 /dose). Data from Study I and II were pooled to generate a combined dataset of 52 patients. Because genotype could not be determined in four individuals, the final genomic data included 48 patients.

For analyzing the effect of *CYP3A4* gene polymorphism, data from patients carrying at least one *CYP3A4*1B* allele were compared against those from patients that were homozygous for *CYP3A4*1* allele. Similarly, patients with either *CYP3A5*1/*1* or *CYP3A5*1/*3* (*CYP3A5* expressers) were pooled and compared against patients with *CYP3A5*3/*3* (*CYP3A5* non-expressers). To assess the effect of *ABCB1* 3435C>T, patients with T/T or C/T were grouped together and compared against patients with C/C.

Dose-normalized trough and 2-hour post dose concentrations of tacrolimus, 13-DMT and 15-DMT were significantly lower in patients carrying at least one *CYP3A4*1B* allele (Table 3). Similarly, the values of tacrolimus C_0 /dose and C_2 /dose were significantly lower in *CYP3A5* expresser as compared with *CYP3A5* non-expressers (Table 3).

Six out of eight patients carrying at least one *CYP3A4*1B* allele were *CYP3A5* expressers. When the combined effect of *CYP3A4* and *CYP3A5* gene on C_0 /dose and C_2 /dose of tacrolimus and metabolites was examined, it was observed that *CYP3A4*1B* carriers-*CYP3A5* expressers had significantly lower concentrations as compared to *CYP3A4*1/*1*-*CYP3A5* non-expressers and *CYP3A4*1/*1*-*CYP3A5* expressers (Table 3).

The effect of genetic polymorphism on metabolite to parent concentration ratio

Patients with *ABCB1 3435 C/C* had significantly lower metabolite to parent concentration ratio for 13-DMT and 15-DMT at either trough (C_0) or 2-hour (C_2) post dose (Table 4). The metabolite to parent concentration ratio at C_0 was also lower in *CYP3A4*1B* carrier but only for 15-DMT. *CYP3A5* expresser status did not influence metabolite to parent concentration ratios.

The combined effect of diabetes and gene polymorphism

When C_0 levels of all the patients (33 diabetics and 17 nondiabetics) were analyzed based on the presence of diabetes, we have observed that diabetic patients have significantly higher dose adjusted tacrolimus levels. Data from *CYP3A4*1B* carriers-*CYP3A5* expressers were pooled and compared against others and then were further divided based on the presence of diabetes (Figures 2A-F). Multiple comparison of the groups showed that nondiabetic patients with at least one *CYP3A4*1B* allele and *CYP3A5* expresser have significantly lower tacrolimus C_0 /dose levels of tacrolimus compared to the other three groups (Figure 2A). Moreover, C_0 /dose and C_2 /dose of 13-DMT and 15-DMT were also significantly lower in nondiabetic patients with at least one *CYP3A4*1B* allele and *CYP3A5* expressers compared to some of the other groups (Figures 2C-F).

Discussion

Coexisting pathological conditions such as long-term diabetes may have a significant impact on the concentration of immunosuppressive agents or their metabolites (Akhlaghi *et al.*, 2006; Akhlaghi *et al.*, 2012). Currently, limited data are available regarding the effect of diabetes on the concentration of tacrolimus metabolites. Because of the significance of genetic polymorphism on the tacrolimus disposition (Staatz *et al.*, 2010), the impact of important genetic factors on tacrolimus and metabolite concentrations were studied in conjunction with diabetes status of the patients.

Patients with long-term diabetes exhibit delayed gastric emptying due to diabetes induced autonomic neuropathy that may affect the rate of drug absorption (Dostalek *et al.* 2012a). Longer tacrolimus T_{max} in the patients with diabetes is most likely due to the delayed gastric emptying. Diabetes alters drug protein binding due to elevated protein glycation (Zini *et al.*, 1990). Several clinical studies have investigated the effect of diabetes on CYP3A4 activity. In this regard, Moises *et al.* (2008) have reported reduced clearance of lidocaine (CYP3A4 substrate) in women with gestational diabetes. Moreover, Marques *et al.* (2002) observed that diabetic patients had significantly higher exposure to CYP3A4 substrates, nisoldipine and lidocaine. Protein expression and activity of CYP3A4 was also significantly lower in liver microsomes from diabetic donors than those without diabetes irrespective of CYP3A4

or 3A5 polymorphism; however, CYP3A5 protein expression did not differ (Dostalek *et al.*, 2011a). Similarly, the *in vitro* metabolism of atorvastatin, a CYP3A4 substrate, was lower in liver from diabetic donors in parallel with the increased concentrations of atorvastatin lactone metabolite (Dostalek *et al.* 2012b). Lower CYP3A4 activity in diabetic patients may partly explain the higher AUC₀₋₁₂ of parent tacrolimus observed in the current study. We have observed that diabetic patients have significantly higher tacrolimus and metabolite exposure in the absorption phase (AUC₀₋₅); however, the metabolite to parent AUC ratio was comparable to nondiabetic group.

Tacrolimus has three potential O-linked glucuronidation sites that can form water-soluble glucuronide conjugates prior to elimination (Laverdiere *et al.*, 2011). Carvalho *et al.* (2011) have observed significantly lower glucuronidation capacity and exposure of labetalol, a UGT2B7 and 1A1 substrate, in women with gestational diabetes. Currently, the effect of diabetes on the expression and activity of UGT1A4 is unknown.

In the present study, we have observed that patients carrying at least one *CYP3A4*1B* allele or *CYP3A5* expressers have approximately two times lower C₀/dose than *CYP3A4*1/1* carrier or *CYP3A5* non-expressers. Similarly, several studies have demonstrated that patients with at least one *CYP3A4*1B* allele or *CYP3A5* expressers exhibit significantly lower tacrolimus levels and may need a higher tacrolimus dose to achieve a therapeutic concentration (Staatz *et al.*, 2010). In addition, it is the first time, along with the parent tacrolimus, we have reported significantly lower C₀/dose and C₂/dose for 13-DMT and 15-DMT in patients with at least one *CYP3A4*1B* allele and lower C₀/dose for 15-DMT in *CYP3A5* expressers.

Dai *et al.* (2006) have reported higher metabolite formation of tacrolimus in *CYP3A5* expressers-human liver microsomes; however, we have observed lower 15-DMT levels in *CYP3A5* expressers. This difference can be explained by the complex interplay of efflux transporters and CYP3A enzymes in the human body. In addition, unlike *in vitro* experiments using liver microsomal system, tacrolimus or metabolite concentrations in the systemic circulation equally depend on the hepatic and pre-hepatic enzyme expression and activity.

In this patient population, we have observed that 75% of the patient population (6 out of 8) that express at least one *CYP3A4*1B* allele are also expressing a functional *CYP3A5*. This could be because of a possible linkage between expression of *CYP3A4* and 3A5 genotypes. Hence when the combined effect of *CYP3A4* and *CYP3A5* gene polymorphism on tacrolimus and metabolite levels was assessed, it was observed that *CYP3A5* expresser patients with at least one *CYP3A4*1B* allele have significantly lower C₀/dose and C₂/dose than the rest of the population.

Multiple comparisons of diabetes status and *CYP3A4-CYP3A5* gene (*CYP3A5* expressers with at least one *CYP3A4*1B* allele vs. others) in our study have revealed that non-diabetic patients with at least one *CYP3A4*1B* allele and *CYP3A5* expresser have significantly lower tacrolimus C₀/dose compared to the rest of the study population. However, the sample size was too small to discern a solid conclusion from this observation.

Interestingly, we have observed significantly lower metabolite to parent concentration ratio in patients carrying *ABCB1* 3435C/C allele vs. those carrying 3435C/T, T/T alleles. Patients with *ABCB1* 3435C/C (wild type) are known to have a higher P-gp activity resulting in a reduced exposure to digoxin (a specific P-gp substrate) (Johns *et al.*, 2002). However, we have observed comparable tacrolimus concentrations between *ABCB1* 3435C/C group and 3435C/T, T/T group, but significantly lower metabolite to parent concentration ratio in the *ABCB1* 3435C/C group. This observation suggests that a larger portion of tacrolimus metabolites is effluxed via P-gp after formation in the hepatocyte in patients with *ABCB1* 3435C/C allele that is consistent with the fact that metabolites often are better substrates of transporter proteins (Christians, 2005).

To the best of our knowledge, this is the first time that the disposition of tacrolimus, 13-DMT and 15-DMT, has been reported in relationship with diabetes status and genetic polymorphism. Because diabetic transplant patients in the current study exhibited higher dose-normalized tacrolimus exposure, these patients may need a lower tacrolimus dose in the early post transplant period to reach the therapeutic concentrations.

Conclusions

In conclusion, the observations in the present study provide compelling evidence that diabetes and genetic polymorphism significantly affect the disposition of tacrolimus, 13-DMT and 15-DMT. These findings may have a clinically significant impact on interpretation of the results of therapeutic monitoring of tacrolimus although further studies using much larger sample size are warranted.

Acknowledgments

Partial support of grant # R15 GM101599-01 from National Institutes of Health is gratefully acknowledged. The use of Rhode Island Genomics and Sequencing Centre, supported in part by the National Science Foundation (MRI Grant DBI-0215393 and EPSCoR Awards 0554548 & 1004057), the US Department of Agriculture (Grants 2002-34438-12688, 2003-34438-13111 & 2008-34438-19246), and the University of Rhode Island is acknowledged. Use of the RI-INBRE core facility funded by the NIH grant # P20 GM103430 is gratefully acknowledged.

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Figure 1. (A-D). Concentration-time profiles of tacrolimus and major metabolites in diabetic (N=11) and nondiabetic (N=9) kidney transplant recipients
(A) non dose-normalized profile of tacrolimus **(B)** dose-normalized profile of tacrolimus **(C)** dose-normalized profile of 13-O-desmethyl tacrolimus and **(D)** dose-normalized profile of 15-O-desmethyl tacrolimus. All data are geometric mean and 95% confidence intervals. The open circles represent nondiabetic patients, while the closed circles represent diabetic patients.

Figure 2. (A-F). Effect of diabetes-gene polymorphism on tacrolimus and metabolite concentrations

Dose-normalized trough concentrations of (A) tacrolimus, (C) 13-O-desmethyl tacrolimus, (E) 15-O-desmethyl tacrolimus and 2-hour post-dose concentrations of (B) tacrolimus, (D) 13-O-desmethyl tacrolimus, (F) 15-O-desmethyl tacrolimus in stable kidney transplant recipients separated according to diabetes status (D, ND) and gene polymorphism (*CYP3A4*1B* carrier that are *CYP3A5* expresser or others). *CYP3A4*1B* carrier =

*CYP3A4**1/*1*B* or *CYP3A4**1*B*/*1*B*, D = diabetic, ND = nondiabetic; C_0 = trough concentration C_2 = 2-hour post-dose concentration. P values are based on comparing log transformed values of dose normalized concentrations with univariate ANOVA and pairwise comparisons validated against 1000 bootstrap samples performed using Wild sampling method. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 1
Demographic characteristics of diabetic and nondiabetic patients from Study I and II

Study I patients (12-hour profile)		
	Diabetic (n=11)	Nondiabetic (n=9)
Gender [Male/Female]	11/0	9/0
<i>ABCB1</i> CC/CT+TT	0/9	4/5
<i>CYP3A4</i> *1/*1/*1B+*1B*1B	8/1	8/1
<i>CYP3A5</i> expresser/non-expresser	2/7	4/5
Age [years]	57 (50-62)	48 (42-53)
Weight [Kg]	81.8 (71.4-95.7)	80.7(67.4-94.9)
Diabetes type [1/2]	5/6	NA
Race (C, AA, H, AI) [#]	11, 0, 0, 0	7, 1, 1, 0
Tacrolimus dose [mg]	3.0 (1.5-3.0)	3.0 (2.0-6.5)
Prednisone dose [mg]	5.0 (5.0-7.0)	7.0 (5.0-10.0)
Mycophenolic acid dose [mg]	500 (500-500)	500 (500-1000)
Haemoglobin A1c [%]	7.1 (6.6-8.6)	5.6 (5.2-6.5) *
Glucose [mg/dL]	128 (84-152)	91 (78-109) *
Time post transplantation [months]	14.9 (10.0-29.6)	15.6 (8.0-32.6)
Blood urea nitrogen [mg/dL]	26 (19-34)	19 (14-29)
Creatinine [mg/dL]	1.3 (1.1-1.5)	1.2 (0.9-1.4)
Albumin [g/dL]	4.2 (4.0-4.4)	4.4 (4.1-4.5)
Glycated albumin [%]	1.3 (1.1-1.5)	1.1 (0.8-1.3)
Study II patients (2-hour profile)		
	Diabetic (n=24)	Nondiabetic (n=8)
Gender [Male/Female]	19/5	6/2
<i>ABCB1</i> CC/CT+TT	3/20	1/5
<i>CYP3A4</i> *1/*1/*1B + *1B*1B	19/4	5/2
<i>CYP3A5</i> expresser/non-expresser	5/19	2/4
Age [years]	50 (37-54)	50 (47-63)
Weight [Kg]	84 (73-104)	92 (82-101)
Diabetes type [1/2]	9/15	NA
Race (C, AA, H, AI) [#]	16, 3, 5, 0	3, 1, 3, 1
Tacrolimus dose [mg]	2.0 (2.0-3.0)	4.0 (2.0-6.0)
Prednisone dose (mg)	5.0 (5.0-6.0)	5.0 (5.0-5.0)
Mycophenolic acid dose [mg]	500 (500-750)	500 (500-1000)
Haemoglobin A1c [%]	6.9 (6.2-8.5)	5.7 (5.4-5.8) *
Glucose [mg/dL]	115 (111-181)	96 (89-117) *
Time post transplantation [months]	21 (12-40)	21 (17-50)
Blood urea nitrogen [mg/dL]	19 (16-32)	15 (10-20)

Creatinine [mg/dL]	1.5 (1.1-2.2)	1.0 (0.8-1.5)
Albumin [g/dL]	3.9 (3.7-4.4)	3.8 (3.7-4.2)
Glycated albumin [%]	1.4 (1.2-1.7)	1.5 (1.2-1.6)

All data presented as median (interquartile range);

*

$P < 0.05$

#

C, Caucasian; AA, African American; H, Hispanic; AI, American Indian

Table 2
Summary of pharmacokinetic parameters of tacrolimus and metabolites in diabetic vs. nondiabetic patients from Study I (12-hour profile)

	Diabetic (n=11)	Nondiabetic (n=9)	P value
Parent tacrolimus			
Trough concentration [ng/mL]	6.4 (5.1-7.5)	5.7 (3.8-7.6)	0.475
Trough concentration [ng/mL per mg dose]	2.7 (1.7-3.7)	2.0 (0.8-3.2)	0.320
T _{max} [hour]	3.0 (2.0-4.0)	1.5 (1.4-2.0)	0.012
C _{max} [ng/mL per mg dose]	5.8 (3.8-8.3)	3.2 (2.3-5.2)	0.044
AUC ₀₋₁₂ [ng*hour/mL per mg dose]	37.7 (37.2-63.9)	20.6 (12.9-41.9)	0.037
AUC ₀₋₅ [ng*hour/mL per mg dose]	30.1 (26.4-48.3)	15.1 (10.0-32.2)	0.037
AUC _{5.1-12} [ng*hour/mL per mg dose]	10.8 (8.8-15.2)	5.5 (3.0-11.0)	0.053
Apparent clearance [L/hour/kg]	0.13 (0.08-0.17)	0.28 (0.13-0.58)	0.037
13-O-desmethyl tacrolimus			
T _{max} [hour]	2.1 (2.0-3.0)	1.5 (1.1-2.5)	0.033
C _{max} [ng/mL per mg dose]	0.79 (0.59-6.3)	0.61 (0.2-0.79)	0.110
AUC ₀₋₁₂ [ng*hour/mL per mg dose]	4.7 (2.9-43.0)	2.5 (1.6-4.2)	0.053
AUC ₀₋₅ [ng*hour/mL per mg dose]	3.8 (2.6-35.1)	2.0 (1.2-3.5)	0.053
AUC _{5.1-12} [ng*hour/mL per mg dose]	0.65 (0.55-1.8)	0.4 (0.3-0.8)	0.074
15-O-desmethyl tacrolimus			
T _{max} [hour]	3.1 (2.0-5.0)	3.0 (1.7-3.6)	0.171
C _{max} [ng/mL per mg dose]	0.3 (0.2-3.2)	0.1 (0.08-0.24)	0.014
AUC ₀₋₁₂ [ng*hour/mL per mg dose]	2.4 (1.8-21.0)	1.0 (0.6-2.0)	0.014
AUC ₀₋₅ [ng*hour/mL per mg dose]	1.7 (1.4-16.8)	0.7 (0.5-1.5)	0.014
AUC _{5.1-12} [ng*hour/mL per mg dose]	0.8 (0.5-2.3)	0.2 (0.2-0.6)	0.016

All data presented as median (interquartile range)

AUC₀₋₁₂, area under the concentration-time curve from 0 to 12-hours; C_{max}, maximum concentration; T_{max}, time to reach maximum concentration.

Table 3
The effect of *ABCB1*, *CYP3A4* and *CYP3A5* gene polymorphism on dose-normalized trough (C_0 /dose) and 2-hour post dose (C_2 /dose) concentrations of tacrolimus, 13-O-desmethyl tacrolimus (13-DMT) and 15-O-desmethyl tacrolimus (15-DMT) (data from the Study I and II are combined)

<i>ABCB1</i> genotype			
C_0 /dose	Tacrolimus	13-DMT	15-DMT
3435 C/C (n=8)	1.63 (1.03, 2.57)	0.08 (0.05, 0.12)	0.08 (0.06, 0.11)
3435 C/T + T/T (n=39)	1.86 (1.52, 2.26)	0.17 (0.12, 0.25)	0.15 (0.11, 0.22)
P value	0.59	0.07	0.13
C_2 /dose			
3435 C/C (n=9)	3.57 (2.12, 6.01)	0.35 (0.23, 0.52)	0.12 (0.08, 0.16)
3435 C/T + T/T (n=39)	3.27 (2.51, 4.27)	0.56 (0.36, 0.88)	0.21 (0.14, 0.32)
P value	0.77	0.12	0.03
<i>CYP3A4</i> genotype			
C_0 /dose			
<i>CYP3A4</i> *1/*1B + 1B/1B (n=8)	0.84 (0.46, 1.55)	0.05 (0.03, 0.11)	0.04 (0.03, 0.06)
<i>CYP3A4</i> *1/*1 (n=40)	2.12 (1.82, 2.47)	0.19 (0.14, 0.28)	0.19 (0.14, 0.26)
P value	0.001	0.003	0.0001
C_2 /dose			
<i>CYP3A4</i> *1/*1B + 1B/1B (n=8)	1.21 (0.47, 3.14)	0.13 (0.05, 0.38)	0.04 (0.03, 0.07)
<i>CYP3A4</i> *1/*1 (n=40)	3.99 (3.23, 4.92)	0.71 (0.47, 1.06)	0.27 (0.19, 0.39)
P value	0.006	0.004	0.0001
<i>CYP3A5</i> genotype			
C_0 /dose			
<i>CYP3A5</i> expresser (n=15)	1.16 (0.79, 1.69)	0.12 (0.05, 0.27)	0.09 (0.05, 0.18)
<i>CYP3A5</i> non-expresser (n=33)	2.17 (1.82, 2.54)	0.16 (0.11, 0.22)	0.16 (0.11, 0.22)
P value	0.003	0.120	0.024
C_2 /dose			
<i>CYP3A5</i> expresser (n=14)	1.61 (0.96, 2.69)	0.35 (0.14, 0.91)	0.13 (0.05, 0.33)
<i>CYP3A5</i> non-expresser (n=34)	4.34 (3.41, 5.41)	0.60 (0.34, 0.91)	0.22 (0.15, 0.32)
P value	0.003	0.160	0.098
Combined <i>CYP3A4-3A5</i> genotype			
C_0 /dose			
<i>CYP3A5</i> expresser + <i>CYP3A4</i> *1B carrier (n=6)	0.73 (0.32, 1.68)	0.05 (0.02, 0.14)	0.04 (0.02, 0.06)
<i>CYP3A5</i> expresser + <i>CYP3A4</i> *1/*1 (n=9)	1.57 (1.13, 2.18)*	0.21 (0.07, 0.67)	0.17 (0.07, 0.43)*
<i>CYP3A5</i> non-expresser + <i>CYP3A4</i> *1B carrier (n=2)	1.29 (NA)	0.6 (NA)	0.04 (NA)
<i>CYP3A5</i> non-expresser + <i>CYP3A4</i> *1/*1 (n=30)	2.33 (1.96, 2.77)*	0.18 (0.13, 0.25)	0.18 (0.13, 0.26)*
P value	0.0001	0.031	0.002

$C_2/dose$			
<i>CYP3A5</i> expresser + <i>CYP3A4*1B</i> carrier (n=5)	0.83 (0.31, 2.24)	0.11 (0.03, 0.45)	0.04 (0.02, 0.07)
<i>CYP3A5</i> expresser + <i>CYP3A4*1/*1</i> (n=9)	2.33 (1.33, 4.07)*	0.66 (0.19, 2.27)	0.27 (0.08, 0.88)*
<i>CYP3A5</i> non-expresser + <i>CYP3A4*1B</i> carrier (n=2)	3.16 (NA)	0.19 (NA)	0.06 (NA)
<i>CYP3A5</i> non-expresser + <i>CYP3A4*1/*1</i> (n=32)	4.63 (3.77, 5.70)*#	0.72 (0.47, 1.12)*	0.27 (0.19, 0.39)*
P value	0.0001	0.026	0.003

All data are presented as geometric mean (95% confidence interval); NA, due to limited sample size data is not available;

*, data significantly different from *CYP3A5* expresser + *CYP3A4*1B* carrier group;

#, data significantly different from *CYP3A5* expresser + *CYP3A4*1/*1*; *CYP3A5* expresser, *CYP3A5*1/*1* or *CYP3A5*1/*3*; *CYP3A5* non-expresser, *CYP3A5*3/*3*; *CYP3A4*1B* carrier, *CYP3A4*1B/*1B* or *CYP3A4*1/*1B*.

Table 4
The effect of *ABCB1*, *CYP3A4* and *CYP3A5* gene polymorphism on the metabolite to parent concentration ratio at the trough (C_0) and 2-hour post dose (C_2). (data from Study I and II are combined)

	13-DMT/Tacrolimus	15-DMT/Tacrolimus
<i>ABCB1</i> genotype		
<i>Pre-dose</i>		
3435 C/C (n=8)	0.05 (0.04, 0.06)	0.05 (0.04, 0.06)
3435 C/T + T/T (n=39)	0.09 (0.06, 0.13)	0.08 (0.06, 0.12)
P value	0.002	0.008
<i>Two-hour post dose</i>		
3435 C/C (n=8)	0.10 (0.08, 0.12)	0.03 (0.03, 0.04)
3435 C/T + T/T (n=39)	0.17 (0.12, 0.25)	0.07 (0.05, 0.10)
P value	0.01	0.002
<i>CYP3A4</i> genotype		
<i>Pre-dose</i>		
<i>CYP3A4</i> *1/*1 (n=40)	0.09 (0.06, 0.13)	0.08 (0.06, 0.12)
<i>CYP3A4</i> *1/*1B + 1B/1B (n=8)	0.06 (0.04, 0.08)	0.05 (0.04, 0.06)
P value	0.34	0.004
<i>Two-hour post dose</i>		
<i>CYP3A4</i> *1/*1 (n=40)	0.18 (0.12, 0.25)	0.07 (0.05, 0.10)
<i>CYP3A4</i> *1/*1B + 1B/1B (n=8)	0.11 (0.07, 0.18)	0.04 (0.02, 0.06)
P value	0.28	0.15
<i>CYP3A5</i> genotype		
<i>Pre-dose</i>		
<i>CYP3A5</i> expresser (n=15)	0.10 (0.05, 0.20)	0.08 (0.05, 0.14)
<i>CYP3A5</i> non-expresser (n=33)	0.07 (0.05, 0.10)	0.07 (0.05, 0.10)
P value	0.33	0.86
<i>Two-hour post dose</i>		
<i>CYP3A5</i> expresser (n=15)	0.22 (0.12, 0.39)	0.09 (0.05, 0.16)
<i>CYP3A5</i> non-expresser(n=33)	0.15 (0.10, 0.21)	0.05 (0.04, 0.08)
P value	0.27	0.20

All data are presented as geometric mean (95% confidence interval) 13-DMT, 13-O-desmethyl tacrolimus; 15-DMT, 15-O-desmethyl tacrolimus