Impact of genetic polymorphism of *ABCB1 (MDR1) 2677G>T* –A in kidney donors on tacrolimus level in Jordanian kidney transplant recipients during the early post transplantation period

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Abstract: The aim of this study was to determine the role of donors' *ABCB1 G2677T/A* polymorphism on tacrolimus dose requirements, trough levels and dose-adjusted trough concentrations among Jordanian renal transplant recipients during the early, unstable period post transplantation. Donors of those renal transplant recipients who were started on tacrolimus post-transplantation (n=53) were genotyped for *MDR1 G2677T/A* using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis. Tacrolimus doses (mg/kg body weight), trough concentrations (ng/ml), dose-adjusted trough concentrations (ng/ml per mg/kg body weight) were compared among patients according to donors' allelic status for *MDR1* (*G2677T/A*). Among the 53 donors, 28 (52.8%) were carriers of *GG*, 20 (37.7%) of *GT*, and 5 (9.4%) of *TT MDR1* alleles. Trough tacrolimus concentrations in recipients of donors carrying at least one *T* mutant alleles (*2677TT* or *2677GT*, serine phenotype) did not differ significantly from trough concentration in recipients of donors carrying homozygote wild, *2677GG* genotype (alanine) during the early 6 months post renal transplantation (P = 0.40, 0.62, 0.42, 0.60, 0.93, 0.66 for months 1-6, respectively). In conclusion, donor's *MDR1* gene polymorphism has no impact on trough tacrolimus concentration during the early period post-transplantation. To date, the results of studies remain controversial and many other factors must be considered to predict variability profile of trough tacrolimus levels accurately.
Keywords: *Tacrolimus, dose requirements, trough levels, dose-adjusted trough concentrations*

1. Introduction

Tacrolimus is widely used as a primary immunosuppressive agent in patients after organ transplantations. However, it is difficult to maintain an appropriate blood concentration of tacrolimus because of a narrow therapeutic index and remarkable intra- and inter-individual variability (Uesugi et al., 2006). Since a low blood concentration is one of the factors responsible for acute rejection, while a high blood concentration induces adverse effects, such as hypertension, hyperglycemia, and nephropathy (Li et al., 2006), close monitoring of tacrolimus blood level is essential to achieve the optimal immunosuppressive effect and to limit its toxicity (Wei-lin et al., 2006), and daily doses must be adjusted according to its whole blood trough concentrations. Achieving therapeutic trough levels is of paramount importance during the period immediately after transplantation (Roy et al., 2006). The inter-individual variations are thought to be caused by different factors including genetic factors (Kapturczak et al., 2004; Yu et al., 2006). Some genetic polymorphism in biotransformation enzymes or transporter proteins, such as cytochrome P450 iso-enzymes and P-glycoprotein encoded by ATP-

binding cassette sub-family B member 1 (ABCB1, also called MDR1) genes in donors and/or recipients, appear as important determinants of tacrolimus blood pharmacokinetics (Wavamunno and Chapman, 2008). Tacrolimus is a substrate for P-glycoprotein (P-gp), the product of the multidrug resistance-1 (MDR1) gene. Some of the single nucleotide polymorphisms (SNP) of MDR1 reported correlated with the in vivo activity of P-gp (Anglicheau et al., 2003), Prospective studies are needed to explore this field and improve utility of donor and recipient genotype testing in managing immunosuppressant therapy (Wavamunno and Chapman, 2008).

Up to date there is no study on the influence of donors MDR1 genetic polymorphism on tacrolimus

dose requirements in Jordanian kidney recipients during the early period post transplantation.

2. Material and Methods

T The study was approved by the Local Research Ethics Committee of the King Hussein Medical Centre (KHMC), Consequent kidney transplant recipients treated with tacrolimus and attending regularly kidney transplant clinic at KHMC during the first six months post transplant and their corresponding donors were provided informed consent to participate in this study.

Blood samples were obtained from 53 Jordanian kidney transplant donors whose recipients were treated with tacrolimus. Blood was collected in EDTA-tube for genotyping analysis and stored at 4oC until DNA extraction. All procedures of tacrolimus concentration measurements were performed in the clinical laboratory of the KHMC. Whole blood samples from patients were drawn during hospital stay post transplantation or during routine visits to nephrology outpatient clinic for drug concentration measurements. The concentrations were obtained directly at the laboratory. Trough concentration of tacrolimus was measured in whole blood by IMx Tacrolimus II assay which utilizes micro particle enzyme immunoassay (MEIA) in the Abbott IMx system. Blood levels were reported for all patients and dose-adjusted concentrations were calculated by dividing the concentration by the corresponding total daily dose on milligram per kilogram basis. Commercial kit (Wizard genomic DNA purification kit, Promega), was used to extract genomic DNA from the whole blood samples.

PCR-Restriction Enzyme Assays was performed to detect the allele's variant in the present study. PCR product was digested by Ban I or Rsa I restriction endonucleases according to the enzyme manufacturer recommendations.

Genotype frequencies for ABCB1 G2677T/A allele among Jordanian population were estimated from the results of PCR-RFLP test for kidney transplant donors.

Data set was tested for normality of distribution using Kolmogorov-Smirnov Z test. All values were represented as means (±standard deviations). For statistical analysis based on different genotype groups, independent-samples t-test was used. Wild genotypes were compared against the combined group of homozygote mutant genotype and heterozygote mutant genotype. All calculations were performed with SPSS 17.0 for windows. P value < 0.05 was considered as statistically significant.

3. Results

The phylogenic trees were constructed based on nucleotide sequences and amino acid sequences of merRgene. In figure 1A and 1B Evolutionary relationships of 19 taxa has been shown.The evolutionary history was inferred using the Neighbor-Joining method. The 58 kidney transplant recipients and 53 corresponding kidneys donors participated in the study; the remaining 5 donors were not available because there was no opportunity to contact them. All patients in this study were of the same ethnic group (Caucasians).

The mean tacrolimus trough concentrations, tacrolimus weight-adjusted doses and tacrolimus dose-adjusted trough levels during the first 6 months post kidney transplantation are shown in Table 1. During the first 3 months post-transplant, mean tacrolimus trough concentrations were higher than the target level (7-10 ng/ml); progressively declining towards month 4, after which the average tacrolimus trough concentrations remained stable within the target level. Tacrolimus doses were progressively decreasing over six months post transplantation. Tacrolimus dose-adjusted trough levels were steadily increasing till the third month post transplantation after which they remained relatively stable.

Among 53 kidneys transplant donors, 28 (52.8 %) were homozygote wild type (GG), 20 (37.7%) were heterozygote mutant (GT) and the remaining 5 (9.4%) were homozygote mutant type (TT).

Dose-adjusted trough level (ng/ml per mg/kg/body weight), daily dose (mg/kg body weight), and trough concentration (ng/ml) of tacrolimus were compared among recipients of donors with different allelic status of MDR1 G2677T/A: recipients of donors carrying the GG genotype (homozygous wild type) and of those carrying at least one T allele (TT or GT) (homo- and heterozygous mutant type, respectively). Tacrolimus trough level, dose and dose-adjusted level did not differ significantly between different MDR1 G2677T/A genotype groups during the first 6 months post transplant as presented in Table 2.

4. Discussions

The clinical use of tacrolimus is complicated by its narrow therapeutic range and highly variable pharmacokinetics among various individuals. Some patients do not reach target concentrations using recommended initial doses of tacrolimus. They, therefore, have an increased risk of under immunosuppression and acute rejection during the early period post organ transplantation. Several have shown the importance of SNPs of the MDR1 gene in response to drugs (Ling-Na et al., 2011; Yan et al., 2011; Munshi, 2012; Vivona et al., 2012). The

association of the ABCB1 gene SNP with tacrolimus dose requirements has been recognized as a genetic basis for the observed inter individual differences in pharmacokinetics (Macphee et al., 2002; Anglicheau et al., 2003; Hesselink et al., 2003; Tada et al., 2005). In our study, MDR-1 alleles frequency (G allele: 72%, T allele: 28%) is consistent with data previously published for Caucasian population (Haufroid et al., 2004; Mourad et al., 2005; Gonzalez

Table 1. Mean tacrolimus concentrations, doses and dose-adjusted levels during the first six months in Jordanian kidney transplant recipients (n=58); (*Paired t-test, compared to the previous month)

Parameter, Mean ± SD (Range)	Treatment period post transplantation (months)					
	1 (n=55)	2 (n=58)	3 (n=56)	4 (n=55)	5 (n=48)	6 (n=51)
Tacrolimus trough	15.27±4.72 (8.65-30)	13.18±3.27 (8.2-20.33)	10.56±2.33 (6.77-20.45)	9.95±2.37 (6.6-15.7)	9.94±2.39 (5.8-16)	9.21±2.44 (4.9-19)
*P**		0.001	<0.001	0.02	0.84	0.041
Tacrolimus weight-adjusted	0.19±0.03 (0.11-0.27)	0.15±0.05 (0.05-0.29)	0.11±0.05 (0.04-0.23)	0.095±0.04 (0.03-0.18)	0.083±0.04 (0.03-0.18)	0.077±0.04 (0.03-0.17)
*P**		<0.001	<0.001	<0.001	<0.001	<0.001
Tacrolimus dose-adjusted	83.91±33.02 (39.4-175.1)	102.86±51.78 (34.3-238.5)	118.3±70.25 (36.1-287.2)	126.32±64.4 (44.3-360)	145.8±78.5 (54-341)	146.1±82.01 (46.3-374.5)
*P**		0.001	0.006	0.299	0.021	0.511

Table 2. Relation between donor's *MDR1 G2677T/A* genotype (n=53) and tacrolimus trough level, dose and dose-adjusted level during the first 6 months post transplantation (***** Homozygote wild type, ******mutant (homo & heterozygote carrying at least one *T*); (******* t- test)

Month	**Tacrolimus pharmacokinetic parameter, mean ± SD**								
	Trough level (ng/ml)			Weight-adjusted dose (mg/kg/day)			Dose-adjusted trough level (ng/ml per mg/kg/day)		
	Donor's genotype			Donor's genotype			Donor's genotype		
	*GG**	*TT/GT***	*P****	*GG**	*TT/GT***	*P****	*GG**	*TT/GT***	*P****
1	14.6±3.6 (n=26)	15.7±5.7 (n=24)	0.40	0.18±0.03	0.19±0.02	0.67	81.9±28.6	86.8±38.9	0.62
2	13.0±4.0 (n=28)	13.5±2.6 (n=25)	0.62	0.15±0.05	0.15±0.05	0.91	101±52.3	108.5±55.8	0.62
3	10.3±1.8 (n=27)	10.9±3.03 (n=24)	0.42	0.12±0.05	0.10±0.05	0.24	106.5±63.3	134.9±81.3	0.17
4	10.1±2.4 (n=27)	9.7±2.4 (n=23)	0.60	0.1±0.04	0.09±0.05	0.51	116.5±52.8	138.8±81.3	0.25
5	9.95±2.2 (n=24)	10.02±2.6 (n=20)	0.93	0.09±0.04	0.08±0.04	0.51	132.6±56.9	169.7±100	0.13
6	9.05±2.04 (n=26)	9.4±2.98 (n=20)	0.66	0.08±0.04	0.08±0.04	0.90	136.1±64.6	164.9±106.1	0.26

et al., 2008; Innocenti et al., 2009; Provenzani et al., 2009).

Genetic polymorphisms of MDR1 may be important for tacrolimus pharmacokinetics since P-gp, the MDR1 product, is an important transport protein known to be involved in tacrolimus absorption in the gut, distribution across the body, metabolism and excretion (Li et al., 2006). It was reported that the G allele of G2677A/T polymorphism was associated with a higher expression level of P-gp in the placenta compared with other genotypes (Tanabe et al., 2001).

An important relation was noted for the exon 21 G2677T/A SNP in 81 renal transplant recipients, most of whom were Caucasian: tacrolimus dose requirement was 40% higher and the concentration/dose ratio 36% lower in homozygous mutant than wild-type carriers in renal transplant recipients at 1 month post transplantation (Anglicheau et al., 2003). Another study found that recipients carrying the (T or A) mutation had tacrolimus concentrations 44.7% higher than that in the wild-type individuals (Mendes et al., 2009).

In 86 Chinese renal transplant recipients, the MDR1 G2677T/A and C3435T gene polymorphisms were correlated with the whole blood concentration of tacrolimus, and in order to obtain similar blood concentration, patients with 2677GG and 3435CC genotype carriers should take the drug at a higher dose than those with 3435CT, 3435 TT, or 2677 TT at three, six, and twelve months post transplantation (Wang et al., 2005). Among 3435T allele carriers, a weak association was noted between recipients' ABCB1 polymorphism in exon 26 and tacrolimus dose requirements at 3 months after renal transplantation (Macphee et al., 2002).The homozygous carriers of the 3435T allele showed on average more than a twofold lower intestinal P-gp expression level compared to the CC genotype (Haufroid et al., 2004). It was emphasized a need for prospective studies to explore the impact of genetic polymorphism of transport proteins in kidney donors that play an important role in recipient's drug pharmacokinetics (Wavamunno and Chapman, 2008). Such studies are anticipated to improve utility of donor and recipient genotype testing in managing immunosuppression therapy.

The present study found a no significant relationship between G2677T/A donor's genotype and tacrolimus trough concentration in kidney transplant recipients during the early post-transplant period. Tacrolimus dose-adjusted trough concentration was not significantly different between patients who obtained kidney from donors with at least one mutant T allele and those who obtained kidney from donors with wild type allele. The mean tacrolimus blood levels during month 1, 2 and 3 post transplantation were (15.27±4.72, 13.18±3.27, 10.56±2.33 ng/ml, respectively) being above the recommended range of (7-10 ng/ml), while during month 4, 5 and 6 levels were (9.95±2.37, 9.94 ±2.39 and 9.21±2.44 ng/ml, respectively) within the recommended range. Our results show that the mean doses required to achieve target levels of tacrolimus during the early 6 months were (0.19, 0.15, 0.11, 0.095, 0.083 and 0.077 mg/kg/day, respectively) steadily decreasing during the first six months post transplantation. During the first 3 months post transplant doses were within recommended range (0.15–0.3 mg/kg/day) (Staatz and Tett, 2004) and very similar to doses used in European populations, (0.12 and 0.168 mg/kg/day) (Margreiter, 2002; Thervet et al., 2008), or American population (0.1 mg/kg/day) (Macphee et al., 2002). However, doses during the period of 4-6 months in our study were lower than the recommended dose.

In conclusion, the impact of the kidney donors MDR1 2677G>T/A polymorphism on tacrolimus pharmacokinetics is insufficient to have a significant effect on clinical outcome of Jordanian renal transplant patients during early post transplant period. To establish the predictive role of the donor's MDR1 gene polymorphism in tacrolimus pharmacokinetics, there is a need for a large multicenter prospective trial assessing the impact of different individual SNPs and haplotypes in a standard fashion in renal transplant patient population with corresponding donors that is uniformly treated and systematically evaluated at different periods post transplantation.

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