

Influence of *SLCO1B1* and *SLCO2B1* Polymorphisms on Tacrolimus Pharmacokinetics and Clinical Response

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Abstract

Background: Immunosuppressant such as tacrolimus have narrow therapeutic range and are often associated with increased risk of nephrotoxicity in individuals that receive this drug after renal transplantation. Variants in transporters genes have been associated with variability in plasma concentration of tacrolimus and higher risk of adverse effects. Our aim was to investigate the effect of *SLCO1B1* (c.388A>G, c.521T>C) and *SLCO2B1* (c.-71T>C) variants on the efficacy and safety of tacrolimus immunosuppressive therapy in kidney transplant recipients.

Methods: *SLCO1B1* and *SLCO2B1* polymorphisms were detected by TaqMan genotyping and were associated to tacrolimus pharmacokinetics and incidence of acute rejection or diarrhea.

Results: Carriers of the allele *SLCO1B1* c.388G had lower dose adjusted blood concentration (CO/D) of tacrolimus when compared to 388AA carriers, while *SLCO1B1* c.521T>C had no effect. Carriers of CC genotype of *SLCO2B1* c.-71T>C SNP had higher CO/D of tacrolimus when compared to TT carriers. When we consider the effect of the haplotype (c.388A>G and c.521T>C) of *SLCO1B1* on tacrolimus CO/D and incidence of rejection, carriers of *SLCO1B1* *1b haplotype had lower CO/D and lower incidence of rejection when compared to wild type haplotype *1a (p>0.05).

Conclusions: *SLCO1B1* and *SLCO2B1* polymorphisms can contribute for a more safety immunosuppressive treatment in kidney recipients.

Keywords: Polymorphisms; Pharmacokinetics; Renal transplant; SLCO; Tacrolimus

Introduction

In 2009 the number of patients in the waiting list for kidney (34 640), liver (4304), kidney/pancreas (576), heart (305) and lung (161) transplants had grown from 44 to 188% during the previous 7 years [1]. However, the number of transplants performed on Brazil has grown in the last years. Comparing the total of transplants made in 2001 and 2011, it can be noted an increase of 59%. From the total of 6839 transplants performed in 2011 the majority was of kidney (4957) followed by liver (1388) [www.abto.org.br].

The biggest challenge faced by organ transplant is rejection. Immunosuppressive therapy has been used successfully to increase patient survival by reducing incidence of graft rejection and/ or drug-related toxicity. Nonetheless, individual response of organ transplant recipients differs among immunosuppressive drugs using standardized dose regime. Among factors claimed to influence individual responses to drug therapy are disease state, kidney and liver functions, hormonal levels, pharmacokinetic drug-to-drug interactions, diet, life style, and genetic variation [2].

Tacrolimus is a critical-dose immunosuppressive drug that acts preventing the activation of T lymphocytes inhibiting their immune response. It has a narrow therapeutic index and significant inter- and intra-individual variability in blood concentrations under a fixed-dose regimen. Studies have shown that high plasma concentrations of tacrolimus are related with adverse effects, such as nephrotoxicity, neurotoxicity, hypertension and diabetogenic effects [3]. Therefore, it is important to monitor tacrolimus levels in order to adjust the dose and to provide the benefit of the immunosuppressive treatment.

Genetic polymorphisms in membrane transporters can cause change in their expression or conformation, leading to different individual responses to drugs, which can affect the efficacy or safety of the therapy [4,5].

Several polymorphisms in genes encoding the solute carriers (SLC) have been described, specifically the organic anion transporting polypeptides (OATPs) OATP1B1 (*SLCO1B1*) and OATP2B1 (*SLCO2B1*). OATPs are the main proteins in the hepatic uptake of drugs of bloodstream, so they are important targets for understanding of therapeutic variability [6].

OATP1B1 has been involved in the drug interaction between rifampin (an OATP1B1 inhibitor) and the immunosuppressive mycophenolate mophetil. When these drugs were co-administered, an increase of mycophenolate acid (MPA) in blood occurred [7]. Because MPA seems to be transported by OATPs, some studies have investigated the effect of polymorphisms in *SLCO1B1*, *SLCO2B1* and *SLCO1B3* in the pharmacokinetics of MPA and its glucuronide metabolite [8-10].

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Tacrolimus was recently recognized as an inhibitor of phalloidin uptake mediated by OATP1B1 [11]. Nonetheless, no previous study has accessed tacrolimus pharmacokinetics in relation to OATPs gene polymorphisms in Caucasians individuals.

Our aim was to investigate the effect of variants of *SLCO1B1* (c.388A>G, c.521T>C) and *SLCO2B1* (c.-71T>C) genes on the efficacy and safety of tacrolimus immunosuppressive therapy in kidney transplant recipients.

Polymorphisms of *SLCO1B1* and *SLCO2B1* genes were detected by TaqMan genotyping and were associated to tacrolimus pharmacokinetics. Carriers of the variant allele *SLCO1B1* c.388G (*1b) had lower dose adjusted blood concentration of tacrolimus and had lower incidence of acute rejection when compared to the other haplotypes (*1a, *5 and *15; p<0.05).

Material and Methods

Subjects and study protocol

Data from 129 patients that underwent kidney transplant from Kidney and Hypertension Hospital – UNIFESP, Sao Paulo, SP, were recorded. All the patients were informed about the study protocol and gave informed consent. The study protocol was approved by the Ethics Committee of the institution.

The eligibility criteria were: (1) age higher or equal to 18 years old; (2) Terminal renal disease, first kidney grafts recipient from living or deceased donor; (3) Donor age less than 65 years old; (4) reactive antibody against a panel.

Patients pregnant or breastfeeding; with positive serology to HIV or HCV; in treatment with aminoglycosides or with the drug under

Variables	Subjects (129)
Receptors	
Age (years)	43 ± 16
Weight (kg)	68.1 ± 13.2
Men	70.5 % (91)
Ethnics	
White	46.5% (60/128)
Mulattoes	36.4% (47/128)
Black	10.8% (14/128)
Others	5.4% (7/128)
Co-morbidities	
Diabetes	13.9% (18)
Hypertension	50.4% (65)
Dyslipidemia	2.3% (3)
BPAR	27.9% (36)
Donors	
Age (years)	44 ± 10
Living donor	58.7% (74/126)
Ethnics	
White	55.3% (68/123)
Mulattoes	34.1% (42/123)
Black	8.1% (10/123)
Others	3.2% (4/123)
Immunosuppression at three-months	
TAC concentration (ng/mL)	5.9 ± 2.7
MPS dose (g/d)	1.4 ± 0.2
PRED dose (mg/d)	5.6 ± 3.0

Note: number of individuals in parentheses. TAC: tacrolimus, MPS: mycophenolate sodium, PRED: prednisone; BPAR: biopsy-confirmed acute rejection

Table 1: Biodemographical and clinical data of the kidney transplant recipients.

investigation or in treatment up to 4 weeks before transplant were excluded from the study.

After kidney transplant the patients received the scheme of treatment tacrolimus (Prograf) + mycophenolate sodium (MPS) + prednisone for 3 months. The first dose of tacrolimus (0.1 – 0.2 mg/kg/day) was administrated up to 48 h after the transplant in equally divided doses. The daily tacrolimus dose was adjusted to reach blood concentration between 5 and 15 ng/mL during two-month therapy. MPS was administered at 720-1440 mg/day in equally doses and prednisone was administered at an initial dose of 30 mg/day and was gradually reduced to a minimum of 5 mg/day.

Samples and laboratory tests

Blood samples were collected after fasting of 10-12 hours, before and after the transplant and during the immunosuppressive therapy. Routine laboratory tests and tacrolimus concentrations were measured after 3, 7, 28, 60 and 90 days after the transplant. Genomic DNA was extracted from the pre-transplant blood sample. Tacrolimus was measured by ARCHITECT assay (Abbott Laboratories).

Genomic DNA extraction

The genomic DNA was extracted using the QIAcube system (Qiagen, Germantown, MD, USA) and quantification was carried out in the Nanodrop ND-1000 (NanoDrop Technologies, Inc., Wilmington, NC, USA). DNA integrity was evaluated by 0.8% agarose gel electrophoresis. The DNA samples were stored at -20°C.

Genotyping by Real Time PCR

SLCO1B1 rs2306283 (c.388A>G) and rs4149056 (c. 521T>C), and *SLCO2B1* rs2851069 (c.-71T>C) polymorphisms were by Real Time PCR, using the TaqMan system and the equipment 7500 Fast (Applied Biosystems, Foster City, CA). The PCR reaction was carried out in a volume of 8 µL, consisting of 4 µL of Genotyping Master Mix (2X), 0.4 L of TaqMan Drug Metabolism Genotyping Assay (20X) and 3.6 µL of DNA (20 ng) diluted in nuclease-free water. Real-time PCR conditions were as follows: an initial phase of 10 min at 95°C, followed by 40 cycles of 15 s at 92°C and 60 s at 60°C. Only in the case of polymorphism *SLCO2B1* c.-71T>C the cycles were extended to 50 cycles and the annealing step was 90 s at 60°C. Genotype calling was performed using the 7500 SDS software (Applied Biosystems).

Statistical analysis

Quantitative variables are the mean value and SEM. Comparisons among genotypes and tacrolimus pharmacokinetics were evaluated by Two-Way ANOVA repeated measures followed by Holm-Sidak method. Chi-square test was used for association studies between polymorphism and incidence of rejection or adverse effects and for all others association tests. Variables with P values less than 0.1 for univariate analysis were considered for possible inclusion in a multivariable analysis. Clinical variables (biopsy-proven acute rejection (BPAR), ethnicity, type of donor and polymorphisms) were considered for possible inclusion in a multivariable analysis.

The results were analyzed using the program SigmaStat for Windows, version 2.03 (SPSS Inc., Chicago/IL EUA). Hardy-Weinberg's equilibrium (HWE) estimation was calculated using the program Court lab. The level of significance established was of p<0.05.

Results

Characteristics of renal transplant recipients

The bio demographical and clinical data of the patients (n=129)

studied are shown in Table 1. The individuals were 43 ± 16 years old and weighted 68.1 ± 13.2 kg. The group was constituted mainly by men (70.5 %), whites (46.5 %) and first living related kidney transplant recipients. The incidence of biopsy-confirmed acute rejection (BPAR) was 27.9%.

All the patients of study were submitted to initial treatment (3 months) of immunosuppression with tacrolimus (TAC) in association with MPS and prednisone. None of the patients was converted to another scheme of immunosuppressive during the study. At 3 months of the immunosuppressive therapy TAC, MPS and Prednisone blood concentrations were 5.9 ± 2.7 ng/mL, 1.4 ± 0.2 g/d and 5.6 ± 3.0 mg/d, respectively. Tacrolimus mean dose decreased from the third day of treatment (0.188 ± 0.003 mg/kg/day) to the second month (0.074 ± 0.003 mg/kg/day). In the first three months after the transplant TAC concentration reduced progressively from 10.13 ± 0.5 ng/mL in the third day to 5.9 ± 0.2 ng/mL in the third month. Dose-adjusted concentration increased from the third day (0.82 ± 0.04 ng.mL⁻¹/mg) to the second month (1.69 ± 0.09 ng.mL⁻¹/mg).

SLCO1B1 and *SLCO2B1* polymorphisms

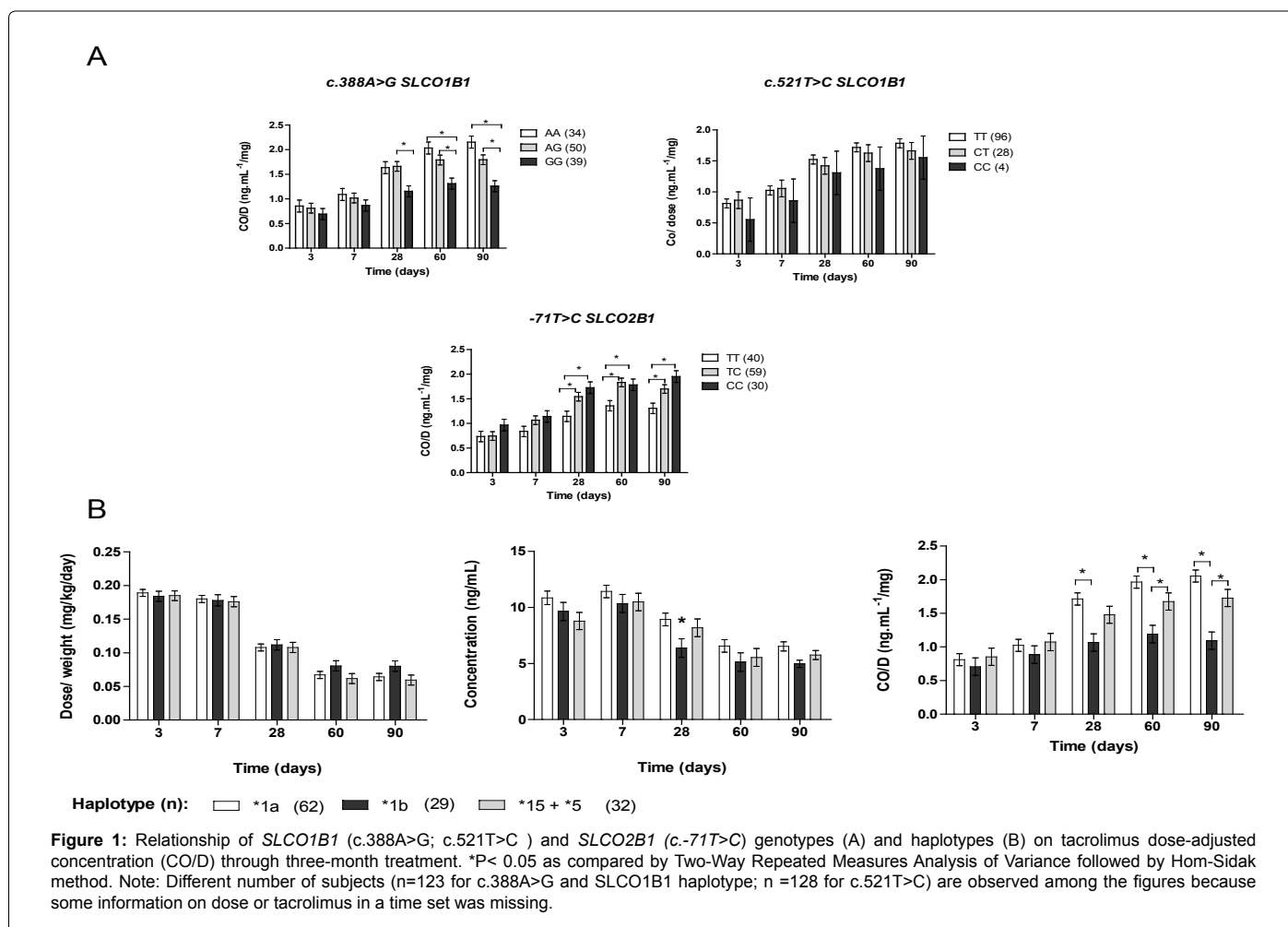
Genotype and allele frequencies were calculated for this sample population. As expected, genotype frequencies for these variants were in Hardy-Weinberg Equilibrium. The frequencies of the two variants alleles (c.388G and c.521C) for *SLCO1B1* was 52% and 14%, respectively. Minor allele frequency (MAF) for *SLCO2B1* -71C allele

was 46%. These frequencies are in accordance to those previously described in Brazilian population [12,13].

SLCO1B1 c.388A>G and c.521T>C single nucleotide polymorphisms (SNPs) were also consistently associated. Haplotypes were defined based on the presence of c.388A>G and c.521T>C polymorphisms alone or in combination, as follows: *SLCO1B1**1a (wild type), *1b (c.388G), *5 (c.521C) or *15 (c.388G and c.521C). The frequencies found for the alleles were *1a (39.0%); *1b (46.5%), *15 (6.3%) and *5 (8.2%).

Relationship of *SLCO1B1* and *SLCO2B1* polymorphisms and tacrolimus pharmacokinetics: The influence of *SLCO1B1* and *SLCO2B1* polymorphisms on administered dose and concentrations of tacrolimus were investigated. Equal administered doses (D) and levels of tacrolimus (CO) were observed among the genotypes of *SLCO1B1* (data not shown). After adjusting concentration (CO/D) of tacrolimus for dose administered, homozygous carriers for *SLCO1B1* c.388G allele presented lower CO/D after 28, 60 and 90 days of therapy compared to AA or AG carriers (Figure 1A). *SLCO1B1* c.521C allele had no effect on tacrolimus pharmacokinetic parameters (Figure 1A).

SLCO1B1 polymorphisms are in linkage disequilibrium as observed before, so we have evaluated the effect of the haplotype on acute rejection. *SLCO1B1**1b presented lower CO/D after 28, 60 and 90 days of TAC therapy and lower TAC blood levels after 28 days (Figure 1B).



Rejection	Genotypes, % (n)			Alleles, %	
<i>SLCO1B1</i>					
c.388A>G	AA	AG	GG	A	G
Yes (36)	36.1 (13)	33.3 (12)	30.6 (11)	52.8	47.2
No (92)	22.0 (20)	44.56 (41)	35.2 (30)	45.1	54.9
	$\chi^2=2.542, 2df, p=0.281$			$\chi^2=0.933, 1df, p=0.334$	
c.521T>C	TT	CT	CC	T	C
Yes (36)	72.2 (26)	25.0 (9)	2.8 (1)	84.7	15.3
No (92)	76.1 (70)	20.6 (19)	3.3 (3)	86.4	13.6
	$\chi^2=, 2gl, p=0.522$			$\chi^2= 0.022, 1df, p= 0.881$	
<i>SLCO2B1</i>					
c. -71T>C	TT	CT	CC	C	T
Yes (36)	33.3 (12)	44.5 (16)	22.2 (8)	44.4	55.6
No (92)	21.7 (20)	47.9 (44)	30.4 (28)	54.3	45.7
	$\chi^2= 0.135, 2gl, p=0.935$			$\chi^2= 1.65, 1df, p= 0.198$	
	Alleles, % (n)				
<i>SLCO1B1</i> haplotype	*1a	*1b	*5	*15	
Yes (70)	48.6 (34)	35.7 (25)	4.3 (3)	11.4 (8)	
No (180)	34.4 (62)	51.7 (93)	9.5 (17)	4.4 (8)	
	$\chi^2=10.85, 3gl, p=0.012$				

Table 2: Relationship of *SLCO1B1* and *SLCO2B1* polymorphisms and biopsy-confirmed acute rejection.

For *SLCO2B1*, a higher CO/D after 28, 60 and 90 days was observed for TC and CC carriers when compared to TT carriers (Figure 1A).

We postulated that *SLCO2B1* and *SLCO1B1* polymorphisms could be associated contributing both to tacrolimus pharmacokinetics. *SLCO2B1* -71T>C was not associated to *SLCO1B1* c.388A>G ($\chi^2= 7.361, 4gl, p=0.118$), however it was associated to *SLCO1B1* c.521T>C SNP ($\chi^2= 12,776, 4gl, p=0.012$). As *SLCO1B1* are consistently associated, we have also compared *SLCO1B1* haplotype and *SLCO2B1* SNP frequencies. *SLCO1B1**1b homozygous (c.388G and c.521T carriers) were associated to *SLCO2B1* -71T allele ($p=0.002$).

Relationship of *SLCO1B1* and *SLCO2B1* polymorphisms and acute rejection and adverse effects: BPAR was observed in 36 kidney transplant recipients. Despite the fact that *SLCO1B1* or *SLCO2B1* polymorphisms affect tacrolimus blood concentrations, there was no difference in the incidence of acute rejection among the genotypes of these polymorphisms (Table 2). However, after we evaluate the effect of the *SLCO1B1* haplotype on acute rejection, the distribution of the positive cases for acute rejection were different among the haplotype alleles *1a, *1b, *5 and *15 ($\chi^2=10.85, 3 df, p=0.012$) (Table 2).

We have performed a multivariate analysis for variables related to biopsy-confirmed renal graft acute rejection, using *SLCO1B1* haplotypes, type of donor (living or deceased donor), tacrolimus blood levels at 28 days after transplant as covariates. Homozygous for *SLCO1B1* *1b allele and carrying one *SLCO1B1**5 allele were independently predictive for lower incidence of rejection (Odds ratio: (*1b/*1b) 0.135 [0.0241-0.755], $p=0.02$; (*5 carriers: 0.182 [0.0289-1.147], $p=0.07$) and living donor was a risk factor for acute rejection (odds ratio: 3.185 [1.186-8.533], $p=0.02$).

The most frequent adverse effect after tacrolimus/ MPS treatment was diarrhea (27.1%), followed by dyslipidemia (7%) and diabetes mellitus (9.3%). Renal dysfunction considered as creatinine clearance < 40 mL/min was observed in only 7 individuals (5.4%) after 3 months of therapy. Then, we evaluate the relationship between polymorphisms and diarrhea in this sample (Table 3). No differences in the incidence of diarrhea was found in *SLCO1B1* c.388G allele or c.521 C allele carriers

Diarrhea	Genotypes, % (n)			Alleles, %	
<i>SLCO1B1</i>					
c.388A>G	AA	AG	GG	A	G
Yes (35)	31.4 (11)	42.9 (15)	25.7 (9)	52.9	47.1
No (94)	24.5 (23)	39.4 (37)	36.2 (34)	44.1	55.9
	$\chi^2=1.383, 2df, p=0.501$			$\chi^2= 1.224, 1df, p=0.268$	
c.521T>C	TT	CT	CC	T	C
Yes (35)	77.2 (27)	17.1 (6)	5.7 (2)	85.7	14.3
No (94)	73.4 (69)	24.5 (23)	2.1 (2)	85.6	14.4
	$\chi^2=1.715, 2df, p=0.424$			$\chi^2=0.034, 1df, p=0.854$	
<i>SLCO2B1</i>					
2B1 -71T>C	CC	CT	TT	C	T
Yes (35)	20.0 (7)	42.9 (15)	37.1 (13)	41.4	58.6
No (94)	23.4 (22)	48.9 (46)	27.7 (26)	52.1	47.9
	$\chi^2=1.069, 2df, p=0.581$			$\chi^2=0.613, 1df, p=0.434$	
	Alleles, % (n)				
<i>SLCO1B1</i> haplotype	*1a	*1b	*5	*15	
Yes (68)	47.0 (32)	39.7 (27)	7.3 (5)	6.0 (4)	
No (186)	35.5 (66)	50.0 (93)	8.1 (15)	6.4 (12)	
	$\chi^2= 2.904, 3df, p=0.407$				

Table 3: Relationship between *SLCO1B1* and *SLCO2B1* polymorphisms and diarrhea.

compared with those carrying the wild -type allele ($p=0.268$). Haplotype analyses, which combined the two alleles (c.388G and c.521C), has also shown no differences in the incidence of diarrhea ($p=0.407$). *SLCO2B1* SNP was also not associated with diarrhea.

Discussion

In this study we have demonstrated that c.388A>G variant of *SLCO1B1* is significantly and independently associated with lower CO/D of tacrolimus in kidney transplant recipients at 1, 2 and 3 months post-transplant. Indeed, in our population, this genetically -determined bioavailability of tacrolimus confers a 20% and 40% lower dose-adjusted concentration of tacrolimus for heterozygous (1.80 vs. 2.15 ng.mL-1/mg) and homozygous for c.388G allele (1.26 vs 2.15 ng.mL-1/mg), respectively. These observations are in accordance with in vivo studies, which described that this variant is associated with higher activity of OATP1B1 in subjects carrying c.388G variant (*1b), resulting in lower bioavailability of pravastatin [14] and pitavastatin [15], and tacrolimus [16]. On the other hand, C allele carriers of *SLCO2B1* -71T>C SNP were associated with higher CO/D of tacrolimus in kidney transplant recipients after 28, 60 and 90 days after transplant. OATP2B1. OATP1B1 uptake transporter is primarily expressed in the liver, whereas OATP2B1 is widely expressed in tissues including the small intestine, liver, and kidney [17]. The effect of the *SLCO2B1* -71T>C variant on OATP2B1 protein is not known, but we may postulate that it could lead to a transporter with impaired uptake activity and this could result in reduced cellular uptake of tacrolimus by kidney. Although, we did not find an association between *SLCO2B1* SNP and tacrolimus toxicity and biopsy-proven rejection. We found that individuals carrying -71C allele were also carriers of *SLCO1B1* c.521C allele, a SNP that leads to an OATP1B1 transporter with impaired function [18], resulting in reduced uptake of Tac by hepatocytes. Thus, the fact that *SLCO2B1* -71C carriers have higher tacrolimus blood levels, could also be attributed to *SLCO1B1* c.521C>C SNP. In our sample, only 4 individuals were carriers of CC genotype for *SLCO1B1* c.521C>C, which may be too low to observe an effect on tacrolimus pharmacokinetics.

Miura et al. [8] investigated the effect of *SLCO1B1*, 2B1 and 1B3

on mycophenolic acid (MPA) and tacrolimus pharmacokinetics in Japanese renal transplant recipients. In their study, the pharmacokinetic parameters of MPA or tacrolimus at 28 days post-transplant were not influenced by *SLCO1B1*, 1B3, or 2B1 genetic polymorphisms. However, in another study by the same group, the phenolic MPA glucuronide (MPAG) dose-adjusted AUC₀₋₁₂ was significantly greater in recipients with *SLCO1B1* 1a/1a, 1a/1b, or 1b/1b than in those with the *15 allele (P=0.0020) [10]. The effect of *SLCO1B1**5 was not investigated, probably because the incidence of c.521C allele on Japanese individuals is very low (0.7%) [19].

The effect of *SLCO1B1* and *SLCO2B1* polymorphisms on tacrolimus safety was also investigated. Lower frequency of *SLCO1B1**5 allele in the group of individuals who had acute rejection and higher *SLCO1B1**1b frequency in the group that had not rejection was observed. Despite the fact this seems to be inconsistent with the low levels of blood tacrolimus concentrations found in c.388G and *SLCO1B1**1b carriers, probably there are other mechanisms and other polyorphisms involved in kidney acute rejection. Capron et al. [20] studying liver transplant patients showed that tacrolimus hepatic concentrations varied markedly among patients and were well correlated with the histological rejection score (Banff score), whereas no correlation was found between the Banff score and trough blood concentrations. It was shown that high tacrolimus hepatic concentrations after the first week of surgery appeared to protect against rejection. In this line, *SLCO1B1**1b carriers have low levels of tacrolimus on blood, however we do not know tacrolimus kidney concentration, which may be are higher in these variant carriers, protecting them from acute rejection.

Diarrhea was the most frequent adverse effect observed, however its incidence was not associated to *SLCO1B1* or *SLCO2B1* SNPs. A relationship between high MPA levels and diarrhea in kidney transplant recipients has been reported [21]. A potential limitation of this study is that we did not measure MPA or MPAG plasma concentration, and then we could not associate MPA levels with *SLCO* polymorphisms. Zucker et al. [22] described higher MPA levels in individuals treated with MMF and tacrolimus when compared to those treated with MMF and cyclosporine. It is feasible to consider that tacrolimus could influence the transportation of MPA or its metabolites mediated by OATP1B1 and it would result in higher MPA/ MPAG plasma concentrations. Accordingly, tacrolimus in OATP1B1 in vitro studies was able to inhibit the transport of phalloidin [11]. In addition, Michelon and collaborators [23], using in vitro experiments, showed that MPA metabolites MPA-phenyl-glucuronide and MPA-acyl-glucuronide are substrates of OATP1B1, and their transport was decreased in the presence of the variant transporter (OATP1B1*5).

In conclusion, carriers of the *SLCO1B1* c.388G and *SLCO1B1**1b haplotype resulted in a lower dose adjusted concentration of tacrolimus and they had lower incidence of biopsy-proven acute rejection when compared to the other haplotypes (*1a, *5 and *15). These genetic variants may contribute for a safer treatment with tacrolimus, however others polymorphism/genes may be involved in tacrolimus efficacy/safety and should be taken into account. Investigations have been limited to small candidate gene studies in allograft recipients and whole genome analysis studies are required to fulfill the genetic determinants of renal transplant outcome.

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References

1. Silva HT Jr, Felipe CR, Abud-Filho M, Garcia V, Medina-Pestana JO (2011) The emerging role of Brazil in clinical trial conduct for transplantation. *Am J Transplant* 11: 1368-1375.
2. Meyer UA (2000) Pharmacogenetics and adverse drug reactions. *Lancet* 356: 1667-1671.
3. Bottiger Y, Brattstrom C, Tyden G, Sawe J, Groth CG (1999) Tacrolimus whole blood concentrations correlate closely to side-effects in renal transplant recipients. *Br J Clin Pharmacol* 48: 445-448.
4. Konig J, Seithel A, Gradhand U, Fromm MF (2006) Pharmacogenomics of human OATP transporters. *Naunyn Schmiedebergs Arch Pharmacol* 372: 432-443.
5. Niemi M, Pasanen MK, Neuvonen PJ (2011) Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. *Pharmacol Rev* 63: 157-181.
6. Kallioikoski A, Niemi M (2009) Impact of OATP transporters on pharmacokinetics. *Br J Pharmacol* 158: 693-705.
7. Goodwin B, Hodgson E, Liddle C (1999) The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module. *Mol Pharmacol* 56: 1329-1339.
8. Miura M, Satoh S, Inoue K, Kagaya H, Saito M, et al. (2007) Influence of *SLCO1B1*, 1B3, 2B1 and *ABCC2* genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. *Eur J Clin Pharmacol* 63: 1161-1169.
9. Miura M, Kagaya H, Satoh S, Inoue K, Saito M, et al. (2008) Influence of drug transporters and UGT polymorphisms on pharmacokinetics of phenolic glucuronide metabolite of mycophenolic acid in Japanese renal transplant recipients. *Ther Drug Monit* 30: 559-564.
10. Picard N, Yee SW, Woillard JB, Lebranchu Y, Le Meur Y, et al. (2010) The role of organic anion-transporting polypeptides and their common genetic variants in mycophenolic acid pharmacokinetics. *Clin Pharmacol Ther* 87: 100-108.
11. Fehrenbach T, Cui Y, Faulstich H, Keppler D (2003) Characterization of the transport of the bicyclic peptide phalloidin by human hepatic transport proteins. *Naunyn Schmiedebergs Arch Pharmacol* 368: 415-420.
12. Rodrigues AC, Perin PM, Purim SG, Silbiger VN, Genvigir FD, et al. (2011) Pharmacogenetics of OATP Transporters Reveals That *SLCO1B1* c.388A>G Variant Is Determinant of Increased Atorvastatin Response. *Int J Mol Sci* 12: 5815-5827.
13. Sortica Vde A, Ojopi EB, Genro JP, Callegari-Jacques S, Ribeiro-Dos-Santos A, et al. (2012) Influence of genomic ancestry on the distribution of *SLCO1B1*, *SLCO1B3* and *ABCB1* gene polymorphisms among Brazilians. *Basic Clin Pharmacol Toxicol* 110: 460-468.
14. Chung JY, Cho JY, Yu KS, Kim JR, Oh DS, et al. (2005) Effect of OATP1B1 (*SLCO1B1*) variant alleles on the pharmacokinetics of pitavastatin in healthy volunteers. *Clin Pharmacol Ther* 78: 342-350.
15. Deng JW, Song IS, Shin HJ, Yeo CW, Cho DY, et al. (2008) The effect of *SLCO1B1**15 on the disposition of pravastatin and pitavastatin is substrate dependent: the contribution of transporting activity changes by *SLCO1B1**15. *Pharmacogenet Genomics* 18: 424-433.
16. Elens L, Capron A, Van Kerckhove V, et al. (2007) 1199G >A and 2677G >T/A polymorphisms of *ABCB1* independently affect tacrolimus concentration in hepatic tissue after liver transplantation. *Pharmacogenetics and Genomics* 17: 873.
17. Kullak-Ublick GA, Ismail MG, Stieger B, Landmann L, Huber R, et al. (2001) Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* 120: 525-533.
18. Tirona RG, Leake BF, Merino G, Kim RB (2001) Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J Biol Chem* 276: 35669-35675.
19. Nozawa T, Nakajima M, Tamai I, Noda K, Nezu J, et al. (2002) Genetic polymorphisms of human organic anion transporters OATP-C (*SLC21A6*) and OATP-B (*SLC21A9*): allele frequencies in the Japanese population and functional analysis. *J Pharmacol Exp Ther* 302: 804-813.

-
20. Capron A, Lerut J, Verbaandert C, Mathys J, Ciccarelli O, Vanbinst R, et al. (2007) Validation of a liquid-chromatography mass spectrometric assay for tacrolimus in liver biopsies after hepatic transplantation: correlation with histopathologic staging for rejection. *Ther Drug Monit* 29: 340.
21. Mourad M, Malaise J, Chaib Eddour D, De Meyer M, Konig J, et al. (2001) Correlation of mycophenolic acid pharmacokinetic parameters with side effects in kidney transplant patients treated with mycophenolate mofetil. *Clin Chem* 47: 88-94.
22. Zucker K, Rosen A, Tsaroucha A, de Faria L, Roth D, et al. (1997) Unexpected augmentation of mycophenolic acid pharmacokinetics in renal transplant patients receiving tacrolimus and mycophenolate mofetil in combination therapy, and analogous in vitro findings. *Transpl Immunol* 5: 225-232.
23. Michelon H, Konig J, Durrbach A, Quteineh L, Verstuyft C, et al. (2010) *SLCO1B1* genetic polymorphism influences mycophenolic acid tolerance in renal transplant recipients. *Pharmacogenomics* 11: 1703-1713.