Combinational Effect of CYP3A5 and MDR-1 Polymorphisms on Tacrolimus Pharmacokinetics in Liver Transplant Patients

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Abstract

Objectives: **Previous studies have reported reduced tacrolimus dose-adjusted exposure in individuals expressing the *CYP3A5*1* allele (reference single-nucleotide polymorphism identification number 776746). However, results on patients from South America are scarce. The objective of this study was to investigate the influence of *CYP3A5* and *MDR1* allelic variants and their correlation on the pharmacokinetics of tacrolimus and a modified release formulation of tacrolimus in stable patients with liver transplant.**

Materials and Methods: **This was a prospective, single center, open-label study. Included patients were ≥ 18 years old and receiving a stable dose of tacrolimus for at least 6 months. Patients receiving stable treatment of twice daily tacrolimus were switched to a once-daily dose of modified release tacrolimus, on a milligram-to-milligram basis, with the modified release formulation administered for at least 4 weeks. Blood levels of tacrolimus were obtained before and 1 month after treatment was switched to the modified release formulation.**

Results: **The frequency of the intron 3 allelic variant of the *CYP3A5* isoform (G-to-A substitution at nucleotide 6986) in recipients was 16.6% and 25% in donors. Dose levels of tacrolimus and the modified formulation were significantly higher in donors and recipients who expressed *CYP3A5* versus donors and recipients who did not express this allele. In addition, patients who received a liver from a donor expressing *CYP3A5* had significantly lower trough concentrations of tacrolimus and the modified formulation. CYP3A5 expression in the donor liver affected tacrolimus (40.46%, *P* = .001) and modified formulation (37.56%, *P* = .001) variability. No asso-ciation was found between the ABCB1 genotype and levels of tacrolimus or its modified formulation.**

Conclusions: **Our data suggest that *CYP3A5*1* in either the donor or recipient resulted in higher mean daily doses of tacrolimus or its modified formulation to achieve target drug exposure in liver transplant patients.**

Key words: CYP3A5 *allelic variants, Liver transplant, Tacrolimus*

Introduction

Tacrolimus is an immunosuppressive drug that has a narrow therapeutic index with high interindividual variation in its pharmacokinetics, leading to difficulties in establishing an empirical dose regimen in organ transplant recipients.[1]

Among the potential causes for large variability, pharmacogenetic studies have suggested an association between *CYP3A5* genotype and tacrolimus pharmacokinetics.[2] It has been reported that a single-nucleotide polymorphism (SNP) within intron 3 of *CYP3A5* (G-to-A substitution at nucleotide 6986,

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*CYP3A5*3* allele) affects the pharmacokinetics of tacrolimus. Only heterozygous or homozygous carriers of the *CYP3A5*1* wild-type allele produce high levels of full-length CYP3A5 mRNA and express high levels of functional CYP3A5 protein (so-called *CYP3A5 expressers*). Previous studies have reported an increase in tacrolimus dose requirements and lower dose-adjusted trough concentrations in heterozygous or homozygous carriers of a *CYP3A5*1* wild-type allele compared with homozygous carriers of a *CYP3A5*3* variant allele.[3] In addition, mRNA expression of the multidrug resistant gene (MDR1) in the intestine is inversely correlated with the concentration-to-dose ratio, that is, the tacrolimus dose needed to obtain a given blood concentration. This gene-dose effect has been suggested in patients who have 1 or 2 mutant alleles in their exon 12, 21, and 26 SNPs, with patients showing lower dose requirements for tacrolimus.[2] However, these studies were conducted with the immediate release, twice-daily formulation of tacrolimus. Tacrolimus is now available as a modified release, once-daily formulation (tacrolimus-XL). Although exposure was demonstrated to be comparable for both formulations, the absorption kinetics was slower with tacrolimus-XL.[4] A possible difference in tacrolimus pharmacokinetics after administration of immediate-release tacrolimus and modified-release tacrolimus might be expected in patients carrying the *CYP3A5*1* allele. Only a few studies have investigated the potential impact of *CYP3A5* allelic variants on tacrolimus-XL and the effects of the allelic variants in patients who switch from previous the immediate release to the modified formulation.

Because tacrolimus is metabolized by both intestinal and hepatic CYP3A5 enzymes, the combined contribution of CYP3A5 expression levels in the native intestine and liver allograft are likely to influence the pharmacokinetics of tacrolimus in liver transplant recipients.[5] Studies to date that have examined the relative influence of donor and recipient CYP3A5 6986A>G genotype in liver transplant recipients have involved only small numbers of patients and hence have been somewhat inconclusive.[3] In this study, our objective was to investigate the influence of intestinal and hepatic *CYP3A5* genotypes and *MDR1* on the pharmacokinetics of tacrolimus-XL after a switch from the immediate-release formulation.

Materials and Methods

This was a prospective, single center, open-label study. We evaluated the correlation between the presence of intron 3 of the CYP3A5 6986G>A isoform and tacrolimus pharmacokinetics after a milligram-to-milligram switch from the twice-daily formulation (Prograf; AstellasPharma, Gador, Argentina) to the new modified-release, once-daily formulation of tacrolimus (Prograf XL; AstellasPharma, Gador, Argentina) in stable liver transplant recipients. All recipients provided their written informed consent for genetic analyses. Immunosuppressive therapy was administered as described in our liver transplant care protocol.

Patients

Patients were recruited within the cohort of liver transplant recipients at our institution. Included patients were ≥ 18 years old and were receiving a stable dose of tacrolimus administered for no less than 6 months.

Other inclusion criteria included the following: primary liver transplant recipients with a full or reduced graft, posttransplant follow-up for no less than 6 months, stable graft function (aspartate aminotransferase, alanine aminotransferase ≤ 3 times the reference, alkaline phosphatase ≤ 5 times the reference value, bilirubin < 3 mg/dL), no biliary or other surgical complications in the 6 months preceding the study, no history of rejection in the 6 months preceding the survey, immunosuppressive therapy with tacrolimus with or without steroids and with or without mofetil mycophenolate, willingness to participate in the study, and ability to sign an informed consent and to adhere to the treatment regimen.

Exclusion criteria included the following: patients who had undergone multiorgan transplant or had received more than 1 liver graft, human immunodeficiency virus infection, intake of drugs that may alter the metabolism or pharmacokinetics of tacrolimus, diagnosis of cancer posttransplant except for patients who had received treatment for basal cell carcinoma, serum creatinine levels > 3 mg/dL, pregnant or breastfeeding women, with women of childbearing age instructed to use birth control, and existence of any medical complication or surgical judgment of the investigator that may interfere with the study objectives.

Treatment

All patients had been receiving a stable dose level of tacrolimus and had received an initial immunosuppressive agent treatment based on tacrolimus, mofetil mycophenolate, and steroids. Patients were given 1500 g/day of mofetil mycophenolate divided into 2 daily doses. The steroid regimen consisted of 1 g intravenous methylprednisolone at time of surgery and 500 mg/day over the next 2 days. This was followed by oral prednisone 80 mg/day, which was progressively reduced to 10 to 20 mg/day for 3 months posttransplant and then to 5 mg/day or suspended because of clinical criteria.

After liver transplant, tacrolimus dosing was adjusted to reach a trough level of between 10 ng/mL and 15 ng/mL within the first 3 months, between 8 ng/mL and 12 ng/mL within the first year, and subsequently to between 5 ng/mL and 7 ng/mL. However, daily tacrolimus dose was adjusted according to the patient's clinical condition, especially in cases of toxicity.

Patients receiving stable treatment with twice-daily tacrolimus were switched to tacrolimus-XL on a milligram-to-milligram basis. The modified formulation was administered for at least 4 weeks.

Blood tests

We obtained blood samples before the morning dose (trough level) and 2 hours after the morning dose of tacrolimus. Samples were then obtained again after the last twice-daily dose of tacrolimus, just before the switch to the once-daily tacrolimus-XL formulation (trough level and hour 2 level), and 4 weeks after the switch to the once-daily modified formulation. Blood samples were collected just before the morning dose of tacrolimus-XL and at 2, 4, and 6 hours after the morning dose.

Tacrolimus pharmacokinetics

Tacrolimus blood concentration was measured with the use of ACMIA immunoassay (Dimension, Siemens Healthcare Diagnostics Inc. Newark, DE 19714 USA, Tacrolumus), according to the manufacturer's instructions. Assay calibration was established using calibrators at 0, 3, 6, 12, 20, and 30 ng/mL, which were tested in duplicate. The lower limit of quantification was 1.2 ng/mL. A cross-validation analysis was performed using high-performance liquid chromatography/mass spectrometry/mass spectometry methodology (R = 0.89).

Collection of patient data

We collected information on patient characteristics (date of birth, sex, weight, and height), posttransplant information, current medications and dose levels, and concomitant medications received by the patient 2 weeks before start of study and during the study. We also collected information about the transplant and donor type, number of transplants, and reason for transplant. Clinical laboratory data included hematology results (hemoglobin, hematocrit, red blood cells, white blood cells and platelets, international normalized ratio). Serological chemistry information was also recorded (creatinine, urea nitrogen, total and direct bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, glutamyl range, and albumin levels).

DNA isolation and genotyping

The CYP3A5 (CYP3A5*1/*3 and *3/*3) and MDR1 (exon 26, cysteine at residue 3435 replaced by threonine [C3435T], exon 21 glycine at residue 2677 replaced by threonine [G2677T]) allelic variants were assessed in posttransplant biopsies from donor liver and blood samples from transplant recipients. Donor's DNA was obtained from 10 cuts with the microtome, thickness of 10 micrometers. The liver biopsies were embedded in paraffin formaldehyde, stored, and preserved immediately after surgery. Liver biopsies were performed on each patient as part of the study protocol.

Genomic DNA extraction from blood and formalin-fixed, paraffin-embedded samples was carried out by QIAamp DNA Blood Mini and QIAamp DNA FFPE kits (QIAGEN, Valencia, CA, USA). The MDR1 C3435T (reference SNP identification number 1045642) and G2677T (reference SNP identification number 2032582) allelic variants were detected using a polymerase chain reaction-restriction fragment length polymorphism assay with the restriction enzymes MboI and RsaI. The CYP3A5*3 (reference SNP identification number 776746) allele was detected by polymerase chain reaction and directly sequenced. Patients who were carriers of this variant (CYP3A5*1/*1 or CYP3A5*1/*3) were selected first and were called "expressing" patients. Recipients carrying the

CYP3A5*3/*3 genotype, responsible for the lack of CYP3A5 expression, were selected second and called "nonexpressing" patients.

Ethical aspects

Patients signed the proper informed consent before collection of study data. The protocol was approved by the Center for Cardiovascular Research and Prevention and the Faculty of Pharmacy and Biochemistry, University of Buenos Aires, ethical committee. All of the protocols conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Statistical analyses

Daily doses of tacrolimus, trough levels and trough levels per dose were estimated according to the expression of the CYP3A5*1 allele between donors and recipients as well as the MDR1 allelic variants. Values are shown as means ± SD, with the two-tailed Mann-Whitney *U* test used to determine the difference in continuous values among groups. The chi-square test was used to analyze differences between expressing and nonexpressing donors and recipients. Multiple linear regression models were made to evaluate the synergistic effect of clinical variables and CYP3A5 genotype on the logarithm of the concentration adjusted for tacrolimus dose. Statistical analyses were performed using STATA version 11.0 statistical software (StataCorp LP, College Station, TX, USA). Significance level was set at $P < .05$.

Results

Our study group included 24 patients greater than 18 years old with a stable dose of tacrolimus administered for at least 6 months after liver transplant who had a planned conversion to the modified once-daily formulation (tacrolimus-XL) by the treating physician. As shown in Table 1, our patient group included mostly men over 50 years old, with all of them from Argentina. Patients had been receiving tacrolimus for no less than 8 months after liver transplant. In addition, more than one-half of our patient group had also been receiving mofetil mycophenolate, and more than one-half had hepatitis C virus-associated hepatocellular carcinoma and alcoholic cirrhosis as the underlying conditions.

Patients were divided into 4 groups based on expression or no expression of the *CYP3A5*1* allele and whether the allele was expressed in each recipient or donor. In recipients, the frequency of the intron 3 polymorphism of the CYP3A5 isoform 6986G>A was 16.6%, with donors showing a frequency of 25% (Table 2). We found no statistically significant deviations in the distribution of allelic variants according to the Hardy-Weinberg test ($P > .05$). Table 3 shows the frequency of the exon 21 and exon 26 allelic variants of the MDR1 gene in recipients.

When we compared recipients by their genotype (those expressing and not expressing CYP3A5*1), the groups were similar regarding demographic

Table 1. Patient Characteristics

Variable	Number (%) or Median (Range)
Male patients	15 (62.5%)
Age (y)	57 (32-67)
Weight (kg)	79.5 (50-95)
Time after transplant (mo)	8 (6-192)
Immunosuppressive therapy	
Tacrolimus-mofetil mycophenolate-prednisone	11 (57.8%)
Tacrolimus-prednisone	6 (31.5%)
Primary disease	
Hepatitis C virus-associated hepatocellular carcinoma	10 (41.6%)
Hepatitis C virus-associated alcoholic cirrhosis	6 (25%)
Cryptogenic cirrhosis	3 (12.5%)
Other*	5 (20.83%)
Pharmacokinetics parameters	
Median tacrolimus concentration (ng/mL)	6.2 (0.6-10)
Median tacrolimus-XL concentration (ng/mL)	6.1 (1.9-12)
Median tacrolimus dose for trough concentration (ng/mL)	119.0 (26.2-425)
Median tacrolimus-XL dose for trough concentration (ng/mL)	116.3 (16.6-370.5)
Median tacrolimus dose (mg/kg/d)	0.047 (0.12-0.3)
Median of tacrolimus-XL dose (mg/kg/d)	0.05 (0.12-0.3)

*Others include hemochromatosis (n = 1; 4.1%), autoimmune hepatitis (n = 2; 8.3%), Budd-Chiari syndrome (n = 1; 4.1%), and alcoholic cirrhosis (n = 1; 4.17%).

Table 2. Distribution of Genotype of CYP3A5 by Recipient, Donor, and Recipient-Donor Combination

Recipient Genotype	Allele	Number (%)
CYP3A5	G/G	20 (83.3%)
	A/G	4 (16.6%)
Donor genotype		
CYP3A5	G/G	18 (75%)
	A/G	6 (25%)
Recipient genotype	Donor genotype	
A/G A/G	2 (50%)	
	G/G	2 (50%)
G/G	A/G	4 (20%)
	G/G	16 (80%)

Table 3. Distribution of Genotype of Phenotype Genotype Project by Recipient and Donor

Recipient Genotype	Allele	Number (%)
ABCB1 1236C<T	C/T	11 (45.8%)
	C/C	10 (41.6%)
	T/T	3 (12.5%)
ABCB1 2677G>T	G/G	9 (40.9%)
	G/T	9 (40.9%)
	G/A and T/A	2 (9.0%)
	T/T	2 (9.0%)

characteristics and primary disease, with no statistically significant differences shown (Table 4). We observed that recipients who expressed the allele needed a higher dosage of tacrolimus and tacrolimus-XL than recipients who did not express the allele. Regarding pharmacokinetics according to genotype of the donor, a clearly lower trough level and higher tacrolimus dose was required in patients who received a liver from donor expressing the allele versus those who received a liver from a donor who did not express the allele (Table 5). The expression of CYP3A5 in the liver from the donor explained the variability in tacrolimus levels (40.46%, $P = .001$) and in the modified formulation (37.56%, $P = .001$). No other clinical variable was statistically significant in the multiple linear regression model. No statistically significant differences in dose versus trough levels were observed between patients who carried the different alleles of ABCB1 (1236C<T and 2677G>T) (Table 6 and Table 7).

Discussion

In this work, we showed the effect of CYP3A5 allelic variants on the pharmacokinetics of tacrolimus. This is the first study in a Latin American population of adult liver transplant patients evaluating this effect in the donor versus recipient in the pharmacokinetics of tacrolimus and tacrolimus-XL.

The CYP3A5 (reference SNP identification number 776746) is a variation of A/G that occurs in intron 3 of the CYP3A5 gene on chromosome 7. If

Table 4. Demographic Characteristics and Pharmacokinetics of Recipients According to CYP3A5 Genotype

Variable	Recipient Genotype G/G	Recipient Genotype A/G
Male patients	12 (60%)	3 (75%)
Age (y)	55.0 (32-67)	61.5 (59-64)
Weight (kg)	77.5 (50-95)	82 (70-85)
Time after transplant (mo)	8 (6-192)	7 (6-10)
Immunosuppressive therapy		
Tacrolimus-mofetil mycophenolate-prednisone	9 (56.2%)	2 (66.6%)
Tacrolimus-prednisone	5 (31.2%)	1 (33.3%)
Primary disease		
Hepatitis C virus-associated hepatocellular carcinoma	8 (40%)	2 (50%)
Hepatitis C virus-associated alcoholic cirrhosis	6 (30%)	0
Cryptogenic cirrhosis	2 (10%)	0
Other (hemochromatosis, autoimmune hepatitis, Budd-Chiari syndrome, alcoholic cirrhosis)	4 (20%)	1 (25%)
Pharmacokinetics		
Median tacrolimus concentration (ng/mL)	5.8 (0.6-10)	7.45 (6-9.7)
Median tacrolimus-XL concentration (ng/mL)	5.5 (1.9-12)	8.15 (6.7-10.3)
Median tacrolimus dose for trough concentration (ng/mL)	129.2 (26.2-425)	97.3 (42-134.1)
Median tacrolimus-XL dose for trough concentration (ng/mL)	139.2 (16.6-370.5)	104.1 (56.7-119.1)
Median tacrolimus dose (mg/kg/d)	0.045 (0.01-0.3)	0.079 (0.07-0.14)*
Median tacrolimus-XL dose (mg/kg/d)	0.045 (0.01-0.3)	0.079 (0.07-0.14)*

Results are number (%) or median (range).
*$P < .05$.

Table 5. Demographic Characteristics and Pharmacokinetics of Donors According to CYP3A5 Genotype

Variable	Donor Genotype G/G	Donor Genotype A/G
Male patients	13 (72)	2 (33)
Age (y)	57 (32-67)	59 (36-70)
Weight (kg)	84 (59-95)	73 (50-81)
Time after transplant (mo)	10 (7-192)	8 (6-117)
Immunosuppressive therapy		
Tacrolimus-mofetil mycophenolate-prednisone	9 (56.2%)	2 (66.6%)
Tacrolimus-prednisone	5 (31.2%)	1 (33.3%)
Primary disease		
Hepatitis C virus-associated hepatocellular carcinoma	9 (50.0)	1 (16.6)
Hepatitis C virus-associated alcoholic cirrhosis	4 (22.2)	2 (33.3)
Cryptogenic cirrhosis	2 (11.1)	1 (16.6)
Other (hemochromatosis, autoimmune hepatitis, Budd-Chiari syndrome, alcoholic cirrhosis)	3 (16.6)	2 (33.3)
Pharmacokinetics		
Median tacrolimus concentration (ng/mL)	5.5 (0.6-10)	5.75 (2.3-9.1)
Median tacrolimus-XL concentration (ng/mL)	5.4 (1.9-12)	5.6 (2.7-10.3)
Median tacrolimus dose for trough concentration (ng/mL)	140.2 (26.2-425)	43 (30-72.9)*
Median tacrolimus-XL dose for trough concentration (ng/mL)	150.7 (48.1-370.5)	52.6 (16.5-119.1)*
Median tacrolimus dose (mg/kg/d)	0.039 (0.01-0.1)	0.105 (0.05-0.3)*
Median tacrolimus-XL dose (mg/kg/d)	0.039 (0.01-0.1)	0.105 (0.05-0.3)*

Results are number (%) or median (range).
*$P < .05$.

Table 6. Demographic Characteristics and Pharmacokinetics Related to ABCB1 1236C<T Genotype

Variable	Genotype		
	C/T	C/C (Wild Type)	T/T
Male patients	8 (72.7%)	6 (60%)	1 (33.33%)
Age (y)	60 (32-67)	51 (35-67)	46.5 (36-57)
Weight (kg)	81 (59-89)	77.5 (60-95)	74 (50-90)
Time after transplant (mo)	8 (6-192)	29 (7-172)	6.5 (6-7)
Immunosuppressive therapy			
Tacrolimus-mofetil mycophenolate-prednisone	8 (80%)	2 (28.5%)	1 (50%)
Tacrolimus-prednisone	2 (20%)	3 (42.8%)	1 (50%)
Primary disease			
Hepatitis C virus-associated hepatocellular carcinoma	6 (54.5%)	3 (30%)	1 (33.3%)
Hepatitis C virus-associated alcoholic cirrhosis	3 (27.2%)	3 (30%)	0
Cryptogenic cirrhosis	0	2 (20%)	1 (33.3%)
Other (hemochromatosis, autoimmune hepatitis, Budd-Chiari syndrome, alcoholic cirrhosis)	2 (18.1%)	2 (20%)	1 (33.3%)
Pharmacokinetics			
Median tacrolimus concentration (ng/mL)	6 (0.6-8.7)	8.5 (2.1-10)	5.7 (4.2-9.1)
Median tacrolimus-XL concentration (ng/mL)	6.3 (1.9-10.3)	6.2 (2.7-11.7)	5.3 (5-12)
Median tacrolimus dose for trough concentration (ng/mL)	110.9 (26.2-369.7)	134.1 (41.4-425)	51.8 (30.3-171)
Median tacrolimus-XL dose for trough concentration (ng/mL)	107.1(47.2-340)	187.9 (48.6-370.5)	65.3 (16.6-360)
Median tacrolimus dose (mg/kg/d)	0.04(0.02-0.14)	0.04 (0.01-0.07)	0.08 (0.03-0.3)
Median tacrolimus-XL dose (mg/kg/d)	0.04(0.02-0.14)	0.04 (0.01-0.07)	0.08 (0.03-0.3)

Results are number (%) or median (range).

Table 7. Demographic Characteristics and Pharmacokinetics Related to ABCB1 2677G>T Genotype

Variable	Genotype			
	G/G Wild Type	G/T	G/A & T/A	T/T
Male patients	5 (55.56%)	5 (55.56%)	2 (100%)	1 (50%)
Age (y)	46.5 (32-64)	60 (55-67)	67 (67-67)	43.5 (36-51)
Weight (kg)	79 (60-90)	81 (59-89)	76 (72-80)	72.5 (50-95)
Time after transplant (mo)	8 (7-172)	10 (6-192)	18-5 (8-29)	22.5 (6-39)
Immunosuppressive therapy				
Tacrolimus-mofetil mycophenolate-prednisone	3 (50%)	6 (85.7%)	1 (50%)	1 (50%)
Tacrolimus-prednisone	2 (33.3%)	1 (14.2%)	1 (50%)	1 (50%)
Primary disease				
Hepatitis C virus-associated hepatocellular carcinoma	4 (44.4%)	4 (44.4%)	1 (50%)	0
Hepatitis C virus-associated alcoholic cirrhosis	2 (22.2%)	2 (22.2%)	1 (50%)	1 (50%)
Cryptogenic cirrhosis	2 (22.2%)	1 (11.1%)	0	0
Other (hemochromatosis, autoimmune hepatitis, Budd-Chiari syndrome, alcoholic cirrhosis)	1 (11.1%)	2 (22.2%)	0	1 (50%)
Pharmacokinetics				
Median tacrolimus concentration (ng/mL)	5.9 (2.1-9.7)	6 (0.6-8.7)	7.3 (5.5-9.1)	7.4 (5.8-9.1)
Median tacrolimus-XL concentration (ng/mL)	6.6 (2.7-12)	5.3 (1.9-10.3)	6.25 (5.9-6.6)	8.3 (5-11.7)
Median tacrolimus dose for trough concentration (ng/mL)	128.6 (41.1-171)	72.9 (26.2-369.7)	185.8 (44-327.6)	107 (30.3-183.6)
Median tacrolimus-XL dose for trough concentration (ng/mL)	140.2 (48.6-360)	100.1 (48.1-340)	142.4 (47.2-237.6)	193.5 (16.6-360.5)
Median tacrolimus dose (mg/kg/d)	0.47 (0.01-0.07)	0.04 (0.02-0.14)	0.076 (0.02-0.125)	0.16 (0.31-0.3)
Median tacrolimus-XL dose (mg/kg/d)	0.47 (0.01-0.07)	0.04 (0.02-0.14)	0.076 (0.02-0.125)	0.16 (0.31-0.3)

Results are number (%) or median (range).

patients are either homozygous for the wild-type allele (CYP3A5*1) or heterozygous for one wild-type, then there is normal splicing of the 13 exons. This results in transcription and production of high levels of mRNA and consequently the metabolizing enzyme for tacrolimus. A point mutation of the CYP3A5 allele G (CYP3A5*3) (A/G) in patients promotes the insertion of an inappropriate exon 3B within the transcript. This exon introduces an early termination codon and produces a nonfunctional protein fragment.[6]

The frequency of patients who express CYP3A5*1 in our study falls between those published in an Asian population (33%-66%) and a white population (9%-15%). These estimates are consistent with previous results from studies in South America in renal transplant patients,[7,8] in which results were from 9% to 27%. These intermediate values between the white and Asian populations reveal the genetic diversity present in Latin America as a mixture of pre-Columbian populations, European whites (present since times of colonialism), Africans (slaves imported by settlers), and later immigrants (mostly from Spain, Italy, France, and Eastern Europe). This mixture has also been represented in pharmacogenetic studies of genes involved in the metabolism of several antineoplastic drugs such as CYP2A6, CYP2D6, CYP2C19, CYP3A4, CYP1A1, CYP2C9, CYP1A1*2C, CYP1A2*1F, CYP3A4*1B, and CYP2D6*2.[9] Although neither our study nor previous population studies in Argentina have the establishment of a population prevalence of CYP3A5 expression as their primary

objective, these findings highlight the importance and need for pharmacogenetic studies to improve the understanding of ethnic variations on metabolism and effect of drugs in Argentina and other Latin American populations. Extrapolating results from other populations may lead to further inaccurate estimates.

CYP3A5 allelic variants from both donor and recipient are associated with changes in the pharmacokinetics of tacrolimus. Our findings are consistent with a recent study published by Uesugi and associates,[10] which retrospectively evaluated the effect of CYP3A5 in 410 adult liver transplant patients over 7 years during the first 5 weeks after transplant. The group observed that the tacrolimus trough level did not change in patients with the same CYP3A5 genotype among those who had received a liver who either had or did not have expression of CYP3A5 alleles, indicating a greater role of CYP3A5 expression in the first month posttransplant. Likewise, Wang and associates studied 96 adult liver transplant patients and found a reduction in the difference for trough level dose between recipients who expressed or did not express the allele even after 2 weeks in contrast to the increase of these differences by donor genotype.[11] In contrast to expression of CYP3A5 in the recipient, the donor's genotype affects the pharmacokinetics of tacrolimus by increasing time. The effect of the expression of CYP3A5 in the recipients is due to intestinal clearance depuration, which is rapidly counterbalanced by an increase in the hepatic clearance. This explains the loss of the clinical significance of the expression of CYP3A5 genotype of the recipient. This result was verified in our adult patients, who were studied 6 months after transplant, in which not only significant differences in the doses of tacrolimus and tacrolimus-XL were found but also in concentration-adjusted dose levels. The expression of this allele in the donor becomes more important after the first few weeks, which is consistent with the idea of recovery, regeneration, and growth of the graft over time. Thus there is an initial inhibition of hepatic metabolism during regeneration of the liver mass, recovery from the insult of ischemia, and reperfusion that explains the delay of the liver in taking control of the metabolism. We found little influence regarding the allelic variants of ABCB1 1236C>T and 2677G>T alleles on concentration, dose, and trough levels of tacrolimus and its modified variant in stable adult patients. When we analyzed the ABCB1 1236C<T genotype, patients who expressed the allelic variant (T/T or C/T) had lower dose-adjusted concentrations and larger doses requirements for tacrolimus and tacrolimus-XL compared with those who expressed the wild-type variant (C/C). These differences were not statistically significant. In addition, in our study of patients according to ABCB1 2677G>T genotype, no differences were evident among those with allelic variants (G/T, T/T, G/A, and T/A) versus those with the wild-type variant (G/G). Although our results may be influenced by the small sample size, our results were consistent with previous studies where the role of these SNPs remains uncertain because of a failure in finding any association or even finding some weak relationship for both ABCB1 1236C<T,12-20 and ABCB1 2677G>T.[12,14,15,17,19-25]

Those who express CYP3A5 often fail to reach the desired target concentrations of tacrolimus, with an increased risk of potentially developing acute organ rejection, especially within the first month posttransplant.[26] When dose requirements increase, dose adjustments are necessary to achieve a specific range that may differ according to the different genotypes of CYP3A5. Knowledge of CYP3A5 genotype in both the donor and recipient appears to be important in understanding the risk of underimmunosuppression over this period.

Conclusions

Our data suggest that expression of CYP3A5*1 in either the donor or recipient resulted in higher mean tacrolimus or the daily-dose formulation to achieve target drug exposure in liver transplant patients.

References

1. Abbasoglu O. Liver transplantation: yesterday, today and tomorrow. *World J Gastroenterol.* 2008;14(20):3117-3122.
2. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet.* 2004;43(10):623-653.
3. Buendia JA, Bramuglia G, Staatz CE. Effects of combinational CYP3A5 6986A>G polymorphism in graft liver and native intestine on the pharmacokinetics of tacrolimus in liver transplant patients: a meta-analysis. *Ther Drug Monit.* 2014;36(4):442-447.
4. Merli M, Di Menna S, Giusto M, et al. Conversion from twice-daily to once-daily tacrolimus administration in liver transplant patient. *Transplant Proc.* 2010;42(4):1322-1324.
5. Mims JL, 3rd, Wood RC. Proportional (fractional) displacement of the Hirschberg corneal light reflection (test): a new easily memorized aid for strabometry; photogrammetric standardization (calibration). *Binocul Vis Strabismus Q.* 2002;17(3):192-198.

6. Kuehl P, Zhang J, Lin Y, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet*. 2001;27(4):383-391.
7. Larriba J, Imperiali N, Groppa R, Giordani C, Algranatti S, Redal MA. Pharmacogenetics of immunosuppressant polymorphism of CYP3A5 in renal transplant recipients. *Transplant Proc*. 2010;42(1): 257-259.
8. Ferraris JR, Argibay PF, Costa L, et al. Influence of CYP3A5 polymorphism on tacrolimus maintenance doses and serum levels after renal transplantation: age dependency and pharmacological interaction with steroids. *Pediatr Transplant*. 2011;15(5):525-532.
9. Roco A, Quinones L, Agundez JA, et al. Frequencies of 23 functionally significant variant alleles related with metabolism of antineoplastic drugs in the Chilean population: comparison with Caucasian and Asian populations. *Front Genet*. 2012;3:229.
10. Uesugi M, Kikuchi M, Shinke H, et al. Impact of cytochrome P450 3A5 polymorphism in graft livers on the frequency of acute cellular rejection in living-donor liver transplantation. *Pharmacogenet Genomics*. 2014;24(7):356-366.
11. Wang Z, Wu S, Chen D, et al. Influence of TLR4 rs1927907 locus polymorphisms on tacrolimus pharmacokinetics in the early stage after liver transplantation. *Eur J Clin Pharmacol*. 2014;70(8):925-931.
12. Tanabe M, Ieiri I, Nagata N, et al. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J Pharmacol Exp Ther*. 2001;297(3):1137-1143.
13. Op den Buijsch RA, Christiaans MH, Stolk LM, et al. Tacrolimus pharmacokinetics and pharmacogenetics: influence of adenosine triphosphate-binding cassette B1 (ABCB1) and cytochrome (CYP) 3A polymorphisms. *Fundam Clin Pharmacol*. 2007;21(4):427-435.
14. Haufroid V, Mourad M, Van Kerckhove V, et al. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenetics*. 2004;14(3):147-154.
15. Mourad M, Wallemacq P, De Meyer M, et al. The influence of genetic polymorphisms of cytochrome P450 3A5 and ABCB1 on starting dose- and weight-standardized tacrolimus trough concentrations after kidney transplantation in relation to renal function. *Clin Chem Lab Med*. 2006;44(10):1192-1198.
16. Fredericks S, Moreton M, Reboux S, et al. Multidrug resistance gene-1 (MDR-1) haplotypes have a minor influence on tacrolimus dose requirements. *Transplantation*. 2006;82(5):705-708.
17. Mourad M, Mourad G, Wallemacq P, et al. Sirolimus and tacrolimus trough concentrations and dose requirements after kidney transplantation in relation to CYP3A5 and MDR1 polymorphisms and steroids. *Transplantation*. 2005;80(7):977-984.
18. Cheung CY, Op den Buijsch RA, Wong KM, et al. Influence of different allelic variants of the CYP3A and ABCB1 genes on the tacrolimus pharmacokinetic profile of Chinese renal transplant recipients. *Pharmacogenomics*. 2006;7(4):563-574.
19. Choi JH, Lee YJ, Jang SB, Lee JE, Kim KH, Park K. Influence of the CYP3A5 and MDR1 genetic polymorphisms on the pharmacokinetics of tacrolimus in healthy Korean subjects. *Br J Clin Pharmacol*. 2007;64(2):185-191.
20. Haufroid V, Wallemacq P, VanKerckhove V, et al. CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. *Am J Transplant*. 2006;6(11):2706-2713.
21. Roy JN, Barama A, Poirier C, Vinet B, Roger M. Cyp3A4, Cyp3A5, and MDR-1 genetic influences on tacrolimus pharmacokinetics in renal transplant recipients. *Pharmacogenet Genomics*. 2006;16(9): 659-665.
22. Renders L, Frisman M, Ufer M, et al. CYP3A5 genotype markedly influences the pharmacokinetics of tacrolimus and sirolimus in kidney transplant recipients. *Clin Pharmacol Ther*. 2007;81(2):228-234.
23. Tsuchiya N, Satoh S, Tada H, et al. Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. *Transplantation*. 2004;78(8):1182-1187.
24. Li D, Lu W, Zhu JY, Gao J, Lou YQ, Zhang GL. Population pharmacokinetics of tacrolimus and CYP3A5, MDR1 and IL-10 polymorphisms in adult liver transplant patients. *J Clin Pharm Ther*. 2007;32(5):505-515.
25. Goto M, Masuda S, Kiuchi T, et al. CYP3A5*1-carrying graft liver reduces the concentration/oral dose ratio of tacrolimus in recipients of living-donor liver transplantation. *Pharmacogenetics*. 2004;14(7):471-478.
26. Chen SY, Li JL, Meng FH, et al. Individualization of tacrolimus dosage basing on cytochrome P450 3A5 polymorphism--a prospective, randomized, controlled study. *Clin Transplant*. 2013;27(3):E272-E281.