The Genotype Frequency of CYP2C19 Enzyme after Liver Transplantation

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ABSTRACT

Background: Liver transplant recipients are treated with various drugs, the metabolism of which is dependent on the cytochrome P450 polymorphic genotype.

Objective: To identify the polymorphic variety of CYP2C19 genotype in liver allograft before and after transplantation.

Methods: The study was conducted on 88 liver recipients. The CYP2C19 genotypes in donors and recipients were the same in 32 and different in 56 recipients. Extracted genomic DNA from the leukocytes and liver graft tissues were analyzed by TaqMan SNP genotyping assay. The distributions of homozygote, heterozygote, poor and ultra-rapid metabolizers' genotypes were investigated in both groups.

Results: The distributions of CYP2C19 genotypes before transplantation in the blood and liver graft were within the normal range. After transplantation, in patients with different CYP2C19 genotype in donors and recipients, the genotypes of homozygote and ultra-rapid metabolizers were significantly decreased (p=0.024); the heterozygotes and poor metabolizer genotypes were significantly increased (p=0.017).

Conclusion: The variety in CYP2C19 genotyping must be considered in patients with different genotypes in donor and recipients to predict the dosage regimens, optimize the treatment and decrease toxicity.

KEYWORDS: Cytochrome P450; Poor metabolizer; CYP2C19; Genotype; Ultra-rapid metabolizer; Liver transplantation

INTRODUCTION

Liver cytochrome P450 enzymes are important enzymes responsible for several drug metabolisms. One of these enzymes is cytochrome P450 2C19 (CYP2C19) with several polymorphisms in its encoding genes [1]. The wild-type CYP2C19*1 allele is associated with a normal function. Two null function variants, CYP2C19*2 and CYP2C19*3 alleles, reduce and the CYP2C19*17 allele increases the catalytic activity of the enzymes, compared with the wild type alleles [2]. Various combinations of these alleles categorize each individual into different classes of CYP2C19 genotypes including homozygous extensive metabolizer (HomEM, CYP2C19*1/*1), heterozygote extensive metabolizer (HetEM, *1/*17, *2/*17, *1/*3*, *1/*2), poor metabolizer (PM, *2/*2, *3/*3), and ultra-rapid metabolizer (URM, *17/*17) (3). Liver transplant recipients (LTRs) are treated with various drugs such as immunosuppressants (tacrolimus), proton pump inhibitors (omeprazole, lansoprazole), and antifungals (voriconazole) [4-7]. The CYP2C19 enzyme activity is essential for metabolism and serum concentration of these therapeutic drugs and outcome of LTRs [5]. According to literature, in donors and recipients with different allelic patterns, donor liver graft does not affect the CYP2C19 genotypes expressed in the peripheral blood of recipients [8]. In case of recipients and donors with different CYP2C19

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genotype, the recipient peripheral blood flows continuously through the new liver graft, and changes the final presentation of CYP2C19 polymorphisms in the liver allograft. These modifications, the so-called homogenous phenomenon, have been assessed in previous studies in relation to different CYP2C19 genotypes (HomEM, HetEM, and PM) [9, 10]. There is however, limited evidence concerning the association between URM genotypes and this phenomenon in LTRs.

This study was conducted to investigate the possible modification in different genotypes of CYP2C19 including HomEM, HetEM, PM and URM among LTRs in the donors and recipients with the same and different CYP2C19 genotypes.

MATERIALS AND METHODS

Study Population and Data Collection

This cross-sectional study was conducted from January 2013 to December 2016 in Namazee Hospital, affiliated to Shiraz University of Medical Sciences, southern Iran. Three types of specimens were examined: blood samples (2 mL EDTA) of donors and recipients collected before the operation (A); liver tissue of the donors taken at the time of transplantation by frozen section (B); and liver tissue of the recipients with clinically suspected acute rejection after transplantation (C). Eighty-eight recipients and their donors were enrolled into this study of whom 176 blood samples and 202 liver tissue sections—including 88 frozen liver tissue (B) before transplantation and 114 liver biopsies (C) after transplantation (1 liver biopsy in 72, 2 in 10, 3 in 2, and 4 in 4 recipients)—were examined. Polymorphisms of CYP2C19 were determined in the blood and liver tissues of both donors and recipients. Demographic data including sex, age and their underlying diseases were collected from the patients' medical records. Acute rejection was defined as elevated liver enzymes after transplantation in recipients with increased up to 3 times the normal limit of total bilirubin, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, and alkaline phosphatase in serum without evidence of biliary complications [11].

Genomic DNA Isolation

The modified method described by Santos, *et al*, was used to isolate DNAs from the specimens [12]. To remove paraffin residues from tissues, pre-warmed xylene (56 °C) was added to each micro-tube (1 mL), kept for 10 min at 56 °C, centrifuged at 15,000 rpm for 10 min; the supernatant was discarded. This procedure was performed in triplicate. The pellets were washed using descending series of ethanol (100%, 95%, 75%). Genomic DNAs were isolated from the extracted pellets using Invisorb® Spin DNA MicroKit III (Invitek, Berlin, Germany) in accordance with the manufacturer's instructions. The quantity and quality of the extracted DNAs were examined spectrophotometrically (Nanodrop ND-1000, Wilmington, USA).

Real-Time PCR Condition

Genotyping was performed using TaqMan Drug Metabolism SNP Genotyping Assays kit (Applied Biosystems, USA), for the following SNPs: G681A (rs4244285), G636A (rs28399504) and C806T (rs12248560) [13]. The final reaction volumes were 25:11.25 µL of template DNA, 12.5 µL of 2X Taqman Universal Master Mix (Applied Biosystems, USA), and 1.25 µL of working stock of SNP genotyping assay. Real-time PCR conditions consisted of an initial denaturation at 95 °C for 10 min, followed by 50 cycles of denaturation at 95 °C for 15 sec and annealing/extension at 60 °C for 90 sec. All the experiments were conducted in duplicate. Real-time PCR was done using ABI 7500 Fast Real-Time PCR System (Applied Biosystems, USA). Polymorphisms of CYP2C19 were classified as follows: HomEM, CYP2C19*1/*1; HetEM, *1/*2, *1/*3, *2/*3, *2/*17; PM, CYP2C19*2/*2, *3/*3 and URM, *17/*17.

Ethics

This study was conducted in accordance with the Declaration of Helsinki as revised in Edinburgh (1975). The study protocol was approved by the Ethics Committee, Shiraz University of Medical Sciences, Shiraz, Iran. Informed writ-

Table 1: Demographic data of the studied participants

Variables	No. of Patients (%)
Donors	
Mean age (yrs)	28 (18–46)
Sex (male:female)	46/42
Recipients	
Mean age (yrs)	42 (18–61)
Sex (male:female)	38/50
Underling diseases	
Viral hepatitis (HBV/HCV)	30/88 (34)
Primary sclerosing cholangitis	18/88 (21)
Wilson's disease	8/88 (9)
Progressive familial intrahepatic cholestasis	6/88 (7)
Budd-Chiari syndrome	4/88 (5)
Autoimmune liver disease	10/88 (11)
Cryptogenic liver cirrhosis	12/88 (14)

HBV: hepatitis B virus, HCV: hepatitis C virus

ten consents were obtained from all participants prior to the blood sampling.

Statistical Analysis

Analysis was done using SPSS® for Windows® ver 18.0 (SPSS Inc, Chicago, Il, USA). Comparison of the frequencies of HomEM, HetEM, PM and URM genotypes in the donors and recipients was performed using χ^2 or Fisher's exact test, whenever necessary. *Student's t* test was used for comparing means between two groups. A p value <0.05 was considered statistically significant.

RESULTS

The mean age of recipients was 42 years. The male:female ratio was 38/50. The mean age of donors, 28 (range 18–46) years, was significantly (p=0.02) lower than that of the recipients (Table 1). Viral hepatitis (hepatitis B and C) was the most common underlying condition in the studied population. The distributions of HomEM, HetEM, PM, and URM genotypes in peripheral blood samples are presented in Table 2. HomEM and HetEM were the most prevalent genotypes in both groups. There was no statistically significant difference in the distributions of four genotypes between donors and recipients in peripheral blood samples (p=0.14). Thirty-two recipients and donors had the same and 56 had different CYP2C19 genotypes in their peripheral blood.

In those with the same CYP2C19 genotypes, no change in the genotype of enzyme in liver graft after transplantation was observed. In those with different CYP2C19 genotypes, however, the genotypes of enzymes were changed in the liver graft after liver transplantation (Table 3). In the latter group, the HomEM and URM genotypes decreased in the recipients (17.9%, 3.6%, respectively), com-

Table 2: CYP2C19 genotypes in the peripheral blood of donors and recipients before transplantation

CYP2C19 Polymorphism	Donors, n (%)	Recipients, n (%)
Homozygote extensive metabolizer	34 (39)	38 (43)
Heterozygote extensive metabolizer	36 (41)	26 (30)
Ultra-rapid metabolizer	10 (11)	16 (18)
Poor metabolizer	8 (9)	8 (9)

Table 3: Differences in the graft liver CYP2C19 genotypes in donors before and after liver transplantation

CYP2C19 Polymorphism	Recipient PBMCs after LT, n (%)	Donor liver graft before LT, n (%)	Donor liver graft after LT, n (%)
Homozygote extensive metabolizer	24 (43)	20 (36)	10 (18)
Heterozygote extensive metabolizer	14 (25)	22 (39)	32 (57)
Ultra rapid metabolizer	12 (21)	8 (14)	2 (4)
Poor metabolizer	6 (11)	6 (11)	12 (21)

PBMCs: peripheral blood mononuclear cells, LT: liver transplantation

pared with those in the donor's graft (35.7%, 14.3%, respectively) (p=0.024). The rates of HetEM and PM increased in the recipients, compared with those in the primary donor's graft (57.1% *vs* 39.3%) and (21.4% *vs* 10.7%), respectively (p=0.017).

DISCUSSION

CYP2C19 is one of the major isoenzymes of human hepatic CYP system. Polymorphism in the enzyme encoding genes influences individual variation of CYP2C19 activity and efficacy [14, 15]. This study investigated the variation of liver graft CYP2C19 polymorphism among donors and recipients with different CYP2C19 genotypes before and after liver transplantation.

In the present study, 32 (36%) of 88 recipients were recognized similar in CYP2C19 genotypes with their donors; 56 (64%) were different. The frequencies of HomEM, HetEM, URM, and PM genotypes in donor' peripheral blood was 39%, 41%, 11%, and 9%, respectively. These rates in other Iranian population were reported 47.5%, 45.9%, 5%, and 1.6% [16]; in Japanese they were 36.6%, 44.6%, 0.0%, and 18.8%, respectively [17]. The frequency of CYP2C19 genotypes are variable and depends on the ethnicity and the number of the study population.

The expression of the final liver graft genotype depends on the donor graft and recipient peripheral blood mononuclear cells, a phenomenon referred to as homogenous phenomenon [8, 9]. In our study, in recipients with different donor genotype of enzyme, HomEM and URM decreased and HetEM and PM increased, compared with those genotypes in the primary donor's graft. Similar results were reported by Chiu, *et al*, when comparing the CYP2C19 polymorphisms of HomEM, HetEM and PM in recipients with the same or different donors' genotypes [9]. Limited studies have investigated URM genotype in the liver grafts.

Patients undergoing liver transplantation receive a wide range of medications. The effectiveness of the post-operative drug therapy is highly influenced by the drug-metabolizing capacity of the donor liver. Variability in the response to treatment, rate of drug metabolism and patient's outcome after transplantation are basically dependent upon the genetic polymorphisms of the drug-metabolizing enzymes such as CYP2C19 [5, 18, 19]. Several studies have shown that the co-administration of proton pump inhibitors with tacrolimus in patients with PM genotype is directly correlated with higher serum concentrations of tacrolimus, leading to adverse reactions and nephrotoxicity [20, 21]. Effective serum level of proton pump inhibitors in LTRs suffering from gastrointestinal ulceration is predictable by CYP2C19 genotyping.

Organ transplantation is concomitant with risk of fungal infections like aspergillosis, candidiasis, and scopulariopsis [7, 22]. Invasive fungal infections were reported in 1%–8% of LTRs with a high mortality rate, ranging from 60% to 80% [6, 7]. Voriconazole is used widely in case of fungal infections, especially invasive aspergillosis and is effective against many of fungi [23-26]. Potential correlations were reported between PM genotype and voriconazole concentration. Increase in the rate of PM leads to increased voriconazole se-

rum concentration and its adverse events [26]. Increased URM genotype, on the other hand, is associated with decreased voriconazole serum level and therapeutic failure among LTRs [26].

The limitation of the present study was limited number of liver biopsies due to inclusion of only some rejected recipients in the study. Invasive methods needed for liver biopsy, might affect the liver transplant outcome; nor is it acceptable by an ethics committee.

In conclusion, changes in the allelic pattern of liver graft after transplantation were observed in the recipients with different donor's genotype. These findings can help clinicians and health care providers to predict changes in cytochrome P450 enzymes and serum drug concentrations in the recipients with new liver grafts and consequently help improve graft outcomes in LTRs.

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