The Role of CYP2C9 Gene Polymorphisms on Anticoagulant Therapy after Heart Valve Replacement

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Key Words

Heart valve replacement · Warfarin · CYP2C9 gene polymorphism

Abstract

Objective: The aim of the present study was to investigate the role of CYP2C9 gene polymorphisms after heart valve replacement in a group of patients on warfarin therapy. **Materials and Methods:** The study population consisted of 74 patients with heart valve replacement. Peripheral blood was collected into evacuated tubes containing EDTA, and DNA was extracted from circulating leukocytes by using a high pure PCR template preparation kit. CYP2C9*2, CYP2C9*3 alleles were detected by using real-time PCR. **Results:** The patients with CYP2C9*1/*3 and CYP2C9*2/*3 genotypes were taking 28.21 and 24.47 mg, respectively, as mean weekly warfarin dose, whereas patients with CYP2C9*1/*1 genotype were taking 33.90 mg. **Conclusion:** The data show that patients with CYP2C9*1/*3 and CYP2C9*2/*3 genotypes needed a lower maintenance dose of warfarin than patients with CYP2C9*1/*1 wild-type genotype.

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Introduction

The commonest therapeutic procedure for the treatment of valvular pathology is the surgical replacement of defective heart valves [1]. Heart valve substitutes are of two principal types: mechanical prosthetic valves and bioprosthetic valves [1]. Despite their satisfactory hemodynamic results and durability, mechanical valves have a substantial risk of systemic thromboemboli and of thrombotic occlusion. Chronic anticoagulation therapy is required in all mechanical valve recipients, which may potentiate hemorrhagic complications [1, 2]. Warfarin is a commonly used oral anticoagulant agent for the prevention of thromboembolic events in patients with mechanical heart valve replacement [3].

Efficacy of warfarin therapy is routinely monitored by the international normalized ratio (INR), which is a ratio of the time required for the patient's blood to coagulate relative to a standardized coagulation time [4–6]. Warfarin's anticoagulant effect is subject to wide interpatient variability, and despite careful dose titration based on evaluation of the INR, the risk of serious hemorrhage during warfarin therapy ranges from 1.3 to 4.2 per 100 patient years of exposure [7]. Although this complication can occur at therapeutic levels, identification of risk factors for the development of a high INR may identify pa-

tients who are at high risk of bleeding. Warfarin therapy mediates its hematological effect predominantly through (S)-warfarin. After a variable half-life, (S)-warfarin is oxidized by cytochrome P-450 2C9 (CYP2C9) to an inactive metabolite that is excreted in bile. Recent pharmacogenetic studies indicate that interindividual sensitivity to warfarin is partly based on the pharmacogenetics of CYP2C9 [8–10]. Two common variant alleles, CYP2C9*2, where cysteine substitutes for arginine at amino acid 144, and CYP2C9*3, where leucine substitutes for isoleucine at residue 359, have been identified. Each of these polymorphisms can occur in a heterozygous or homozygous form, and the presence of both polymorphisms results in a compound heterozygote (CYP2C9*2/*3) [8–10]. The CYP2C9*3 variant is less than 5% as efficient as wild-type enzyme, while CYP2C9*2 shows about 12% of wild-type activity [7–11]. Both of these variants are associated with decreased enzyme activity and hence impaired warfarin clearance [10, 11]. This condition increases the anticoagulation effect of warfarin, thereby leading to a risk of bleeding complications. The determination of CYP2C9 gene polymorphisms in patients with heart valve replacement might be able to help in optimizing warfarin efficacy and minimizing bleeding.

The purpose of the present study was to investigate the effect of CYP2C9 gene polymorphisms on warfarin therapy in a group of patients after heart valve replacement.

Subjects and Methods

The protocol of this study was approved by the Ethics Committee of the School of Medicine, Mersin University, Mersin, Turkey. The patients were from the same geographic region (southern part of Turkey). The study population consisted of 74 patients (46 women and 28 men) from Mersin University Hospital, Turkey, with heart valve replacement who were receiving maintenance warfarin therapy with stable, therapeutic INR values between 2.5 and 3.5 for at least 3 months. Median age was 50.5 years (range 19–75), mean body mass index calculated as kg/m² was 24.3 (range 15.9–34.6). The number of patients with aortic valve replacement was 25, mitral valve replacement 40 and combined aortic valve and mitral valve replacement 9. The warfarin dose was 15–52.5 mg per week. Patients younger than 18 years or those who had liver disease, malabsorption, or long-term diarrheal conditions or were taking medications known to significantly interact with warfarin therapy were excluded from the study.

DNA Extraction and Genotyping of CYP2C9

Peripheral blood for genetic analysis was collected into evacuated tubes containing EDTA. DNA was extracted from circulating leukocytes by using a high pure PCR template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany, catalog No. 1 796 828). CYP2C9*2 and CYP2C9*3 alleles were detected by

using CYP2C9 mutation detection kits on real-time PCR with LightCycler instrument (Roche Diagnostics, catalog No. 3113914).

Hybridization probes 3 and 4 (anchor probes specific for nucleotide positions 430 and 1075, respectively), CYP2C9 enzyme solution (Taq polymerase), CYP2C9 reaction mixture (DNA polymerase reaction buffer, dNTP mix), CYP2C9 control template (heterozygous plasmid DNA), sterile H₂O (PCR grade) were used. For PCR, 10.7 µl H₂O, 2 µl mutation detection mix, 2 µl CYP2C9 reaction mix, 0.3 µl enzyme solution and 50 ng of genomic DNA were taken in a final volume of 20 µl. The cycling programs for CYP2C9 are 1 cycle of denaturation (95°C, 600 s, ramp rate 20°C/s) and 45 cycles of amplification including denaturation (95°C, 10 s, ramp rate 20°C/s), annealing (55°C, 10 s, ramp rate 20°C/s) and extension (72°C, 19 s, ramp rate 3°C/s). After amplification, a melting curve was generated by holding the reaction at 95°C for 60 s (ramp rate 20°C/s) and cooling slowly (ramp rate 20°C/s) to 40°C and then heating to 80°C (ramp rate 0.1°C/s). The final step cooling was 1 cycle 40°C, 30 s (ramp rate 20°C/s).

Measurement of INR

Venous blood was obtained by venipuncture and collected into tubes containing 3.8% sodium citrate (9:1 vol/vol) and plasma separated. Plasma prothrombin times were measured with a Behringer Coagulation System (Dade Behring, Marburg, Germany) using Thromborel S (Dade Behring) as thromboplastin. The INRs were calculated according to the formula $INR = (\text{patient prothrombin time} / \text{geometric mean normal prothrombin time})^{ISI}$. The geometric mean normal prothrombin time was calculated using 20 normal plasmas. The manufacturer's international sensitivity index (ISI) was used for INR calculation.

Statistical Analyses

Factorial covariance was used to define age, sex, and body mass index; basic variance analysis was used to detect the effect of CYP2C9*2 and CYP2C9*3 gene polymorphisms on warfarin dose and INR levels. In all analyses, $p < 0.05$ was considered significant. All statistical calculations were performed using the SPSS software package version 10.0 (Windows SPSS Inc., Chicago, Ill., USA).

Results

There was no association of age, sex and body mass index with CYP2C9 gene polymorphism, warfarin dose and INR ($p = 0.1$). The allele frequencies were 0.69 for CYP2C9*1, 0.13 for CYP2C9*2 and 0.18 for CYP2C9*3. CYP2C9 genotypes is in Hardy-Weinberg equilibrium ($p = 0.88$) and the distribution of CYP2C9 genotypes is given in table 1. The mean warfarin doses and INR are listed in table 2. Patients with CYP2C9*1/*3 and CYP2C9*2/*3 genotypes required a significantly lower maintenance dose of warfarin (28.21 ± 5.14 and 24.47 ± 5.17 mg, respectively) than patients with CYP2C9*1/*1 wild-type genotype (33.90 ± 7.25 mg; $p = 0.028$ for

Table 1. Distribution of CYP2C9 genotypes in patients

Genotype	Frequency	
	n	%
CYP2C9*1/*	48	64.9
CYP2C9*1/*3	7	9.5
CYP2C9*2/*3	19	25.7
CYP2C9*1/*2	0	0.0
CYP2C9*2/*2	0	0.0
CYP2C9*3/*3	0	0.0

The distribution of the genotypes is in Hardy-Weinberg equilibrium ($p = 0.88$). CYP2C9*1/*1 = Wild-type; CYP2C9*1/*3 = heterozygous *3; CYP2C9*2/*3 = heterozygous *2/*3; CYP2C9*1/*2 = heterozygous *2; CYP2C9*2/*2 = homozygous *2; CYP2C9*3/*3 = homozygous *3.

Table 2. Mean ($\pm$ SD) weekly warfarin dose and INR levels for the CYP2C9 genotypes

CYP2C9 genotype	n	Warfarin dose, mg	p value	Mean INR	p value
CYP2C9*1/*1	48	33.90 ± 7.25	ref.	2.32 ± 0.57	ref.
CYP2C9*1/*3	7	28.21 ± 5.14	0.028	2.91 ± 0.29	0.001
CYP2C9*2/*3	19	24.47 ± 5.17	0.000	2.84 ± 0.80	0.015

CYP2C9*1/*3, $p = 0.000$ for CYP2C9*2/*3). Patients with CYP2C9*1/*3 and CYP2C9*2/*3 genotypes had mean INR values significantly higher than CYP2C9*1/*1 wild-type genotype ($p = 0.001$ for CYP2C9*1/*3, $p = 0.015$ for CYP2C9*2/*3).

Discussion

Warfarin therapy is problematic after heart valve replacement due to a narrow therapeutic index coupled with significant interpatient variability in warfarin sensitivity [12]. The CYP2C9 gene encodes the enzyme that catalyzes the conversion of warfarin to inactive metabolites [12, 13]. Any alteration in its activity would, therefore, have an impact on warfarin pharmacokinetics and clinical efficacy. Two common variant alleles of CYP2C9 (CYP2C9*2 and CYP2C9*3) are seen in more than 30% of European and Caucasian populations, but over 95% of Asian and American populations carry the wild-type allele [14]. It is well established that CYP2C9 and VKORC1

polymorphisms make a significant contribution to the interindividual variability in warfarin dose requirement [12, 15]. As a consequence, patients are at risk of developing extreme levels of anticoagulation after receiving standard dosing regimens, particularly during initiation of therapy [13].

In the present study, the frequencies of CYP2C9*1/*1, CYP2C9*1/*3 and CYP2C9*2/*3 are 64.9, 9.5 and 25.7%, respectively. Patients with CYP2C9*1/*3 and CYP2C9*2/*3 alleles showed a decrease of 16.7 and 27.8% in warfarin dose required, compared with patients having the CYP2C9*1/*1 wild-type genotype. Whether these mutations affect functional activity through decreased enzyme expression or altered substrate binding or through other mechanisms remains to be determined [16]. Our findings support the previous suggestion that CYP2C9 gene polymorphisms in patients warrants adjustment of warfarin dose in order to optimize its efficacy and minimize bleeding.

Conclusion

This study shows that patients carrying CYP2C9*1/*3 and CYP2C9*2/*3 need a lower warfarin dose than patients having CYP2C9*1/*1 wild-type genotype. CYP2C9 genotyping before anticoagulant therapy might help to guide warfarin dosage in patients with heart valve replacement, but prospective trials of such protocols are needed to show the real clinical value of genotyping in this situation.

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