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Influence of *CYP2C9* and vitamin k oxide reductase complex (*VKORC*)1 polymorphisms on time to determine the warfarin maintenance dose

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Received September 2, 2010, accepted October 8, 2010

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Pharmazie 66: 222–225 (2011) *doi: 10.1691/ph.2011.0769*

Polymorphisms in cytochrome P450 (*CYP*) *2C9* and the vitamin K oxide reductase complex subunit 1 (*VKORC1*) greatly affect the maintenance dose of warfarin. To prevent adverse events, immediate dose adjustment is required. The purpose of this study was to investigate the influence of these polymorphisms on the time taken to determine the warfarin maintenance dose for individual patients, and to assess the advantages of genotype-based dosing on initial anticoagulant therapy. We analyzed the genotypes of *CYP2C9* and *VKORC1* from 72 patients. The number of days taken to determine the maintenance dose was compared with the genotypes. The time taken to determine the maintenance dose of warfarin in group A (*CYP2C9*1/*1*, *VKORC1 -1639AA*), B (**1/*1*, *− 1639GA*), C (**1/*3*, *−1639AA*), and D (**1/*3*, *−1639GA*) patients was 19 ± 19, 28 ± 28, 27 ± 20 and 7 days, respectively. We analyzed the relationship between the initial dose of warfarin and the number of days required to determine the maintenance dose based on the *VKORC1* genotypes. Patients with the *VKORC1 −1639AA* genotype and who were initially treated with more than 3 mg warfarin, required approximately 2 weeks for the maintenance dose to be determined. Patients with the *VKORC1 −1639GA* genotype and the same initial warfarin dosage required approximately a month; however, patients initially treated with 5 mg of warfarin only required 9.5 ± 5.3 days. We found a tendency that the time taken to determine the warfarin maintenance dose depends on the genotypes. Genotype-based dosing may improve initial anticoagulant therapy.

1. Introduction

Warfarin is the most widely prescribed anticoagulant for the treatment of thromboembolic disorders. As a narrow therapeutic index and large individual variability are observed between the dose of warfarin and its anticoagulant effect (Kaninsky et al. 1997), careful adjustment of the dose based on the prothrombin time (PT), expressed as the international normalized ratio (INR), is essential. There is a sharp increase in the risk of bleeding when the PT-INR exceeds the upper limit of the therapeutic range (Anticoagulants in the Secondary Prevention of Events in Coronary Thrombosis (ASPECT) Research Group 1994, Cannegieter et al. 1995, Fihn et al. 1993), and the risk of thromboembolic events increases when the PT-INR falls below this range (Fihn et al. 1993, Stroke Prevention in Atrial Fibrillation Investigation 1996). To prevent adverse events, an immediate and appropriate dose adjustment of warfarin is required. However, the establishment of a proper maintenance dose is still challenging because of widespread inter-individual variation in response to warfarin. This is explained in part by genetic polymorphisms of the vitamin K oxide reductase complex subunit 1 (*VKORC1*) and cytochrome P450 2C9 (*CYP2C9*).

VKORC1 is the main molecular target of warfarin (Li et al. 2004). This enzyme recycles vitamin K epoxide to the reduced form of vitamin K, an essential cofactor in the formation of the active clotting factors II, VII, IX and X through γ-glutamyl carboxylation. Some studies have shown that the *VKORC1 −1639G>A* polymorphism has a large impact on the maintenance dose of warfarin (Aquilante et al. 2006; Lee et al. 2006; Rieder et al. 2006; Sconce et al. 2005; Veenstra et al. 2005; Wadelius et al. 2005). We have shown that patients with the *−1639AA* genotype require a lower maintenance dose of warfarin than the patients with −1639GA or −1639GG genotype. Multivariate analysis clearly showed that this polymorphism was the most important determinant of the daily warfarin dose, and explained 16.5% of the observed variation (Obayashi et al. 2006).

The *S*-enantiomer of warfarin is predominantly metabolized to 7-hydroxywarfarin by CYP2C9. The enzymatic activity of CYP2C9 has a substantial influence on the observed anticoagulant effects of *S*-warfarin, the primary active form of the drug. Previous findings revealed that the single nucleotide polymorphisms (SNPs) 430C>T (exon 3) in *CYP2C9*2* and 1075A>C (exon 7) in *CYP2C9*3* are common functional variants, which display approximately 70% and 10% of the metabolic capacity of the wild type (*CYP2C9*1*) enzyme, respectively (Furuya et al. 1995; Rettie et al. 1999).

In 2007, the United States Food and Drug Administration (FDA) updated the labeling recommendations for warfarin to stress that genetic information is helpful to improve the estimate of

Table: Population characteristics and relationship between the genotypes and the warfarin maintenance dose

Group	A	B	C	D
CYP2C9	*1/*1	*1/*1	*1/*3	*1/*3
VKORC1 −1639	AA	AG	AA	AG
Number of patients	56	12	3	1
Proportion of males (%)	55	42	100	0
Age (y)	64 ± 13	67 ± 18	66 ± 5.9	52
PT-INR	2.0 ± 0.31	1.9 ± 0.31	1.9 ± 0.49	1.3
Warfarin maintenance dose (mg/day)	3.0 ± 1.2	4.0 ± 1.3†	2.0 ± 1.0	4

PT-INR (Prothrombin time expressed as the international normalized ratio) measured during the maintenance period. Age, PT-INR and Warfarin maintenance dose are expressed as mean ± SD.
† $P < 0.01$, compared with Group A.

warfarin dose for individual patients. Several prospective trials showed the benefit of a genotype-guided warfarin dosage (Anderson et al. 2007; Caraco et al. 2008), and recent studies showed the effects of *CYP2C9* and *VKORC1* polymorphisms on the warfarin response during the initiation of anticoagulant therapy (Limdi et al. 2009; Schwarz et al. 2008). In these studies, the relationship between the genotypes and the control of the PT-INR in the initial therapeutic period was discussed; however, the influence of these two polymorphisms on the time taken to achieve the optimal maintenance dose of warfarin was not clear. The purpose of this study was to clarify the relationship between these two polymorphisms and the time taken to determine the warfarin maintenance dose, and to assess the effectiveness of genotype-based dosing of warfarin in the initial anticoagulant therapy period.

2. Investigations and results

2.1. Population characteristics

Genotype distribution and population characteristics are summarized in the Table. Among these patients, 4 (5.6%) were heterozygous for the *CYP2C9*3* allele; no patients were homozygous for *CYP2C9*3*. The *CYP2C9*2* allele was not detected in any patients. For the *VKORC1 −1639A* allele, 13 (18%) patients were heterozygous and 59 (82%) were homozygous; no patients were homozygous for the *VKORC1 −1639G* allele. These frequencies were similar to those in previous reports on Japanese patients (Kimura et al. 2006; Sconce et al. 2005), and did not deviate from the Hardy-Weinberg equilibrium. Among these groups, there was no significant difference in the warfarin initial doses, the ratio of in-patients / out-patients, age, gender, or the value of the PT-INR after treatment with the maintenance dose of warfarin.

2.2. Relationship between genotypes and warfarin maintenance dose

The relationship between *CYP2C9* and *VKORC1* genotypes and warfarin maintenance doses is summarized in the Table. Group A (*CYP2C9*1/*1*, *VKORC1 −1639AA*) patients required a warfarin maintenance dose of 3.1 ± 1.2 mg/day. Group B (*CYP2C9*1/*1*, *VKORC1 −1639GA*) patients required a significantly higher dose (4.0 ± 1.3 mg/day) of warfarin than group A patients. Group C (*CYP2C9*1/*3*, *VKORC1 −1639AA*) patients required a warfarin maintenance dose of 2.0 ± 1.0 mg/day. The only patient in group D (*CYP2C9*1/*3*, *VKORC1 −1639GA*) required a warfarin maintenance dose of 4.0 mg/day. This relationship between the genotypes and the warfarin maintenance doses was similar to that observed in previous reports (Cannegieter et al. 1995; Fihn et al. 1993; Li et al. 2004; Sconce et al. 2005 Stroke Prevention in Atrial Fibrillation Investigation 1996 Veenstra et al. 2005; Wadelius et al. 2005).

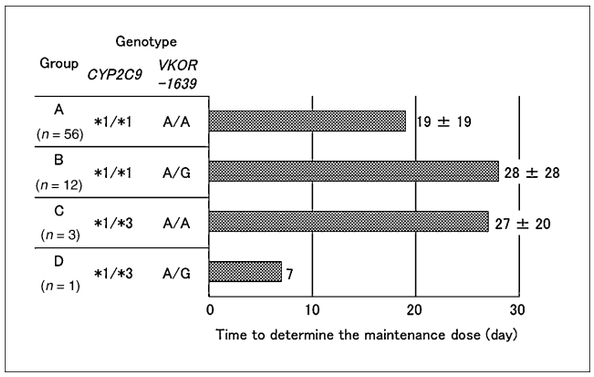

Fig. 1: Relationship between the genotypes and the time taken to determine the warfarin maintenance dose

2.3. Relationship between genotypes and time taken to determine maintenance dose

The relationship between *CYP2C9* and *VKORC1* genotypes and the number of days required to determine the warfarin maintenance dose is shown in Fig. 1. The patients in groups A, B and C required 19 ± 19, 28 ± 28 and 27 ± 20 days, respectively. The patient in group D required 7 days. In this study, the patients who had the genotypes with low frequency (group B and C) required a longer time to determine the warfarin maintenance dose; however there were no significant differences among all groups.

We analyzed the relationship between the initial dose of warfarin and the number of days required to determine the maintenance dose, based on the *VKORC1* genotype (Fig. 2). The patients with

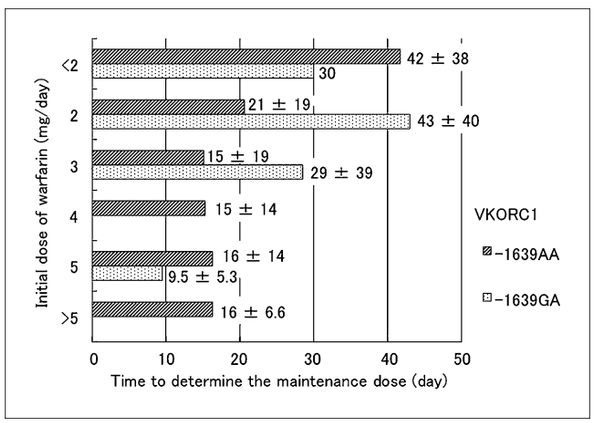

Fig. 2: Relationship between the initial dose of warfarin and the time taken to determine the warfarin maintenance dose compared with the *VKORC1* genotype

the *VKORC1 −1639AA* genotype who were treated with more than 3 mg of warfarin as the initial maintenance dose required approximately two weeks to determine the maintenance dose. Among the patients with the *VKORC1 −1639GA* genotype, patients initially treated with 3 mg of warfarin required 29 ± 39 days to determine the maintenance dose, whereas those patients initially treated with 5 mg of warfarin required 9.5 ± 5.3 days. However, there were no significant differences among these groups.

3. Discussion

Warfarin is the most frequently prescribed drug for the management and prevention of venous and arterial thrombosis. A fixed dose of warfarin for all patients is unachievable because of the highly variable response to warfarin observed in patients. For this reason, the anticoagulant effect of warfarin is evaluated by standardized PT measurements and the dose is adjusted based on the value of the PT-INR. To decrease the risk of bleeding or thrombosis, dose adjustment should be performed appropriately and in as short a term as possible. The significant influence of *VKORC1* and *CYP2C9* on the warfarin maintenance dose is already well documented. However, the benefit of *VKORC1* and *CYP2C9* genotype-guided warfarin dosage on the warfarin response during the initiation of anticoagulant therapy is still not clear.

In this study, we examined the impact of polymorphisms in *VKORC1* and *CYP2C9* on the warfarin maintenance dose and the time taken to determine that dose. The results shown in this study (Table) support the significant influence of the *VKORC1* and *CYP2C9* genes on the maintenance dose of warfarin. On the other hand, although patients with the *VKORC1 −1639GA* or *CYP2C9*1/*3* genotypes required a longer time to reach the maintenance dose than patients with the *VKORC1 −1639AA* and *CYP2C9*1/*1* genotypes, it was not significantly different. The average maintenance dose of warfarin in Japan is 3.3 mg/day (Sconce et al. 2005), if physicians aim for this dose with empirical dosing, it is supposed that it would take more time to adjust the dose in patients with the −1639G allele who require a higher dose or in patients with the *3 allele who require a lower dose. In fact, for patients with the −1639GA genotype, the time taken to achieve the maintenance dose was cut to 9.5 days when an initial dose of 5 mg was chosen. This result strongly suggests that genotype-based warfarin dosing should improve the initiation of anticoagulant therapy.

Recently, interethnic differences in the frequency of the *VKORC1 −1639G>A* polymorphism have been reported (Wadelius et al. 2005; Yuan et al. 2005). In Japanese or Chinese patients (Fihn et al. 1993; Kimura et al. 2006; Sconce et al. 2005; Wadelius et al. 2005; Yuan et al. 2005), the frequency of the −1639AA genotype was approximately 80%, and the frequencies of the −1639GA, −1639AA genotypes were approximately 20% and 1–3%, respectively. This frequency distribution is opposite to that observed in Caucasian populations in which the major genotype is −1639GG (Cannegieter et al. 1995; Li et al. 2004; Stroke Prevention in Atrial Fibrillation Investigation 1996; Veenstra et al. 2005). As shown in our study and previous reports (Cannegieter et al. 1995; Fihn et al. 1993; Kimura et al. 2006; Li et al. 2004; Sconce et al. 2005; Stroke Prevention in Atrial Fibrillation Investigation 1996; Wadelius et al. 2005; Veenstra et al. 2005; Yuan et al. 2005), patients with the -1639AA genotype require a lower warfarin maintenance dose than patients with the -1639GA or -1639GG genotypes. Differences in the distribution of the genotypes have been proposed as the cause of interethnic variability in the warfarin dose requirement (Sconce et al. 2005; Wadelius et al. 2005; Yuan et al. 2005).

As we only identified one patient with the *VKORC1 −1639GA* and *CYP2C9*1/*3* genotypes, we could not perform statistical analysis. Furthermore, there were no patients with the *VKORC1-1639GG* or *CYP2C9*3/*3* genotypes, so the impact of these genotypes could not be estimated and we should carry out further research to clarify these points.

In conclusion, we investigated the relationship between the patient's genotype and the time taken to determine the warfarin maintenance dose. The results suggest that genotype-based dosing of warfarin may improve the initial anticoagulant therapy.

4. Experimental

4.1. Clinical samples

DNA analysis was approved by the Institutional Review Board for clinical investigation and research at Gunma University Hospital and the Ethical Committee for Human Genome Analysis at Gunma University. Written consent was obtained from all participants after they had been informed of the experimental procedure and the purpose of the study.

Two milliliters of blood was obtained from 72 unrelated Japanese patients treated with warfarin at the Department of Cardiovascular Medicine, Gunma University Hospital, Maebashi, Japan. Blood specimens were treated with 4 mg of EDTA-2K to prevent coagulation and analyzed immediately, or stored at −80 °C until assayed.

4.2. Genotyping

The genotyping of *CYP2C9* and *VKORC1* was conducted using the SmartAmp2 assay as previously described (Aomori et al. 2009).

4.3. Data collection

The initial warfarin dose, the date of initiation, subsequent warfarin dose, the date of prescription, age and gender were obtained by reviewing the medical records of the patients who underwent warfarin therapy for the prevention or treatment of thromboembolic disease. The initial dose was determined by the physicians empirically. The maintenance dose of warfarin was defined as the dose which was constant for more than 14 days and was prescribed more than 2 times during this period. The number of days from the initiation of warfarin therapy to the first day when the maintenance dose was prescribed was compared based on the genotypes of *CYP2C9* and *VKORC1*.

4.4. Statistical analysis

The correlations among warfarin dose, *CYP2C9* genotypes, *VKORC1* genotypes and the time taken to determine the warfarin maintenance dose were evaluated by the Kruskal-Wallis H-test and Mann-Whitney U-test with Bonferroni correction. $P < 0.05$ was considered statistically significant. The results are expressed as mean ± standard deviation (SD).

Acknowledgements: This research was supported by the Japan Society for the Promotion of Science, Encouragement of Scientists, 21927001, 2009. We thank Aiko Matsumoto (Gunma University) for secretarial assistance and Tomoko Senbongi (Gunma University) for technical assistance.

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