A vitamin K epoxide reductase-oxidase complex gene polymorphism (–1639G>A) and interindividual variability in the dose-effect of vitamin K antagonists

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Abstract. A daily dose of vitamin K antagonists (VKAs) may vary and its range depends on various interrelated factors. Low responsiveness to VKA (defined as a failure to achieve a target international normalized ratio [INR]) is associated with polymorphisms of the vitamin K epoxide reductase-oxidase complex gene (VKORC1). A highly prevalent promoter single-nucleotide polymorphism (VKORC1-1639 G>A, rs17878363) impairs VKORC1 expression and determines the interindividual variability of the target INR. We studied 57 patients receiving oral anticoagulation, including 50 subjects treated with acenocoumarol (mean dose: 5.7±2.3 mg/day) and 7 treated with warfarin (mean dose: 9.6±4.2 mg/day). The indications for the use of oral anticoagulant therapy were as follows: deep-vein thrombosis (N=23); pulmonary embolism (N=20); arterial thrombosis (N=5); stroke (N=4); atrial fibrillation with transient ischemic attacks (N=2), and history of multiple thromboembolic events (N=3). Identification of the VKORC1 genomic variation was performed using DNA sequencing methods. The prevalence of the mutated allele (VKORC1-1639A) was 41%. The VKORC1-1639G allele carriers required a higher daily dose of acenocoumarol (5.9±1.9 mg) than the noncarriers (4.1±3.3 mg; P<0.001). All of 5 low responders (who failed to achieve a target INR using standard dose requirements of VKAs) were homozygous for the 1639G allele. Low responders did not differ from good responders with respect to age, gender, and body mass index. Our findings suggest the potential benefits from pharmacogenetic testing, and provide evidence that the VKORC1-1639 G>A gene polymorphism may explain at least in part the low responsiveness to acenocoumarol.

Keywords: antivitamin K, coumarin resistance, human, pharmacogenetics, polymorphism, thromboembolism.

Vitamin K antagonists (VKAs), including warfarin, acenocoumarol and phenprocoumon, are highly effective in the prevention and treatment of thromboembolic disorders. Acentocoumarol (acenocoumarin – the 4’-nitro analog of warfarin) is the most commonly used VKA in clinical practice in Poland for the prevention and treatment of venous and arterial thromboembolic disorders (Sawicka-Powierza et al. 2008). Acenocoumarol, like warfarin, exerts its anticoagulant effects by inhibition of vitamin K epoxide reductase-oxidase complex (VKORC1) subunit 1 activity. The effectiveness and safety of oral anticoagulation is critically dependent on maintaining the prothrombin time, expressed as the international normalized ratio (INR). The guidelines recommend a target INR of 2.5 for long-term oral anticoagulant therapy with VKA in the secondary prevention of venous thromboembolism (VTE), ischemic stroke, and atrial fibrillation (Ansell et al. 2008). A number of studies have shown an increased risk of thromboembolic events when an INR value falls below 2 (Fihn et al. 1993), especially in elderly patients (Hackam et al. 2005). A daily dose of acenocoumarol may vary from 2 mg to 40 mg, and the range depends on various environmental factors, such as diet (Martini et al. 2007), seasons (Salobir et al. 2002), medications, or on individual features of patients, such as age, body weight, ethnicity (Blann et al. 2004), and genotype.
A complete or partial resistance to VKA is infrequent and is mostly associated with \textit{VKORC1} gene polymorphisms. According to warfarin dosage adjustment, Rieder et al. (2005) categorized the common \textit{VKORC1} gene variation into 2 main haplotypes: group A (haplotypes H1 and H2), related to the low VKA dose requirement, and group B (haplotype H7-H9), related to a higher dose requirement. For the European population, Geisen et al. (2005) arranged 3 haplotypes (VKORC1*2, VKORC1*3 and VKORC1*4), explaining over 99% of its genetic variability. The haplotype VKORC1*2 includes several single-nucleotide polymorphisms (SNPs), affecting both coding and noncoding regions of the \textit{VKORC1} gene. The main SNPs: rs17878363, rs9934438, rs9934438, rs2359612 (called M2, M17, M18 and M19, respectively) are clustered in the VKORC1*2A haplotype, and they are responsible for a decreased (about 50%) expression of the \textit{VKORC1} gene and a higher required dose of warfarin (D’Andrea et al. 2005; Geisen et al. 2005). A promoter SNP (\textit{VKORC1} -1639 G>A, rs17878363), which impairs VKORC1 enzyme expression (Rieder et al. 2005), is highly prevalent in European cohorts (40%) (Geisen et al. 2005). It is also useful to foresee the total variability of warfarin dose: patients carrying the –1639G allele required a higher dose of warfarin compared with carriers of the –1639A allele (Sconce et al. 2005).

Our study was designed to investigate the contribution of the common VKORC1*2 SNP (–1639G>A) in the modulation of VKA (mainly acenocoumarol) response in respect of interfering factors like age, body mass index (BMI), and disease treated. We evaluated the effect of the –1639G allele on the INR values in relation to the daily dose of anticoagulants in patients on long-term VKA treatment, largely those taking acenocoumarol. Moreover, we analyzed the relation between the –1639G>A alleles and the mean daily dose of acenocoumarol or warfarin prescribed to achieve the target anticoagulation intensity.

We recruited 57 adult patients aged 47.4±13.5 years (29 women and 28 men), receiving oral anticoagulant therapy for at least 6 months. Male and female patients do not differ with respect to age (46.2±12.4 vs. 44.5±14.6 years; \( P = 0.41 \)) and BMI (24.5±3.1 vs. 25.4±3.9 kg m\(^{-2}\); \( P = 0.21 \)). Fifty patients were treated with acenocoumarol in a daily dose between 1 mg and 10 mg (mean dose: 5.7±2.3 mg/day), while 7 patients received warfarin in a daily dose ranging from 5 mg to 15 mg (mean dose: 9.6±4.2 mg/day). The percentage of males, age, BMI, and INR were similar in patients receiving acenocoumarol and warfarin: 50% vs. 43% (\( P = 0.73 \)); 47.6±14.0 vs. 46.0±10.2 years (\( P = 0.44 \)); 25.1±3.7 kg m\(^{-2}\) vs. 24.1±2.4 kg m\(^{-2}\) (\( P = 0.27 \)); and 2.4±0.39 vs. 2.1±0.48 (\( P = 0.34 \)), respectively. All eligible patients had an objectively confirmed episode of VTE, ischemic stroke or arterial thrombosis, and had stable anticoagulation for at least 2 previous months. In order to transform the warfarin dose to that of acenocoumarol, we used the transition factor between 2 drugs of 1.85 (warfarin dose was divided by 1.85) according to the algorithm published by Rosendaal’s group (van Leeuwen et al. 2008). The indications for the use of oral anticoagulant therapy were as follows: deep vein thrombosis (\( N = 23 \)); pulmonary embolism (\( N = 20 \)); arterial thrombosis (\( N = 5 \)); stroke (\( N = 4 \)); atrial fibrillation with transient ischemic attacks (\( N = 2 \)), and history of multiple thromboembolic events (\( N = 3 \)). Exclusion criteria were: (1) known cancer or another severe concomitant disease; and (2) use of drugs interfering with VKA metabolism, such as barbiturates, carbamazepine, and rifampicin. All subjects were advised to refrain from products containing large amounts of vitamin K. Written informed consent was obtained from patients for participation in the study, genetic investigation, and processing of their data. A target INR rate was set above 2 and was defined as the last INR result available. The INR values were determined by BCS automated analyzer (Siemens Healthcare Diagnostics). The “low responsiveness” to VKA was defined as a failure to achieve a target INR using acenocoumarol or warfarin standard dose requirements in the absence of known dose-increasing factors within the previous 3 months.

Genomic DNA was extracted from blood samples, and identification of \textit{VKORC1} genomic variation was performed using a set of primers flanking the \textit{VKORC1} gene promoter region: sense (5’-CAAGTTCCAGGGATTCATGC-3’) and antisense (5’-CCAAGCACGGCTAGACCCAATG-3’) (Geisen et al. 2005). The 555-bp PCR products were purified by the enzymatic digestion method (Exonuclease I, \textit{E.coli} and Shrimp Alkaline Phosphatase – SAP, Fermentas, Vilnius, Latvia) and sequenced using a BigDye Terminator Cycle Sequencing Kit with an ABI Prism 310 Genetic Analyzer (Applied Biosystems) according to manufacturer’s instructions. DNA sequencing analysis was performed using SeqScape v2.5 software. The reference sequence is registered in