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International Journal of Clinical Pharmacology & Toxicology (IJCPT)
ISSN 2167-910X

VKORC1 (-1639) Polymorphisms do not Affect Long-Term Stability of Anticoagulation with Warfarin

Research Article

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Abstract

The extent of INR fall following vitamin K supplementation to patients on warfarin is related to *VKORC1-1639G>A* genotype, with those carrying the *GG* genotype demonstrating a significantly larger fall in INR compared to those carrying the *GA*, and *AA* genotype. We hypothesized that, due to the expected variability in dietary vitamin K intake over time, patients with the *VKORC1(-1639)GG* polymorphism could have poorer anticoagulation control than *GA* or *AA* carriers. 234 atrial fibrillation (AF) patients on warfarin therapy were studied. Anticoagulation control was assessed by the determination of percentage time in therapeutic range (% TTR) of INR, the frequency of INR monitoring and warfarin dose changes over a 12 months period. There were no significant differences between *GG GA* and *AA* variants in mean % TTR (66, 61, 68), mean number of INR monitoring events (12.4, 13.5, 13.0) and mean warfarin dose changes (3.9, 4.0, 3.3), respectively. The study results failed to confirm our hypothesis that patients with the *VKORC1(-1639) GG* variant have poorer anticoagulation control, indicating that the effect of any such influence at the pharmacological level is too small to influence clinically relevant outcomes in our population.

Keywords: Warfarin; Anticoagulation; Stroke; Vitamin K; Diet; *VKORC1* Polymorphism.

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Received: October 20, 2015
Accepted: November 17, 2015
Published: November 19, 2015

Citation: Abohelaika S, Kamali F, Wynne H (2015) *VKORC1 (-1639)* Polymorphisms do not Affect Long-Term Stability of Anticoagulation with Warfarin. *Int J Clin Pharmacol Toxicol,* 4(6) 192-194. **doi: http://dx.doi.org/10.19070/2167-910X-1500032**

Introduction

Achieving a stable anticoagulant effect with vitamin K antagonists such as warfarin is important as they have a narrow therapeutic range, with risk of bleeding or thrombosis increasing with over and under-anticoagulation respectively. Outcomes for patients in atrial fibrillation anticoagulated for thromboembolic prophylaxis improve with more time spent within the desired therapeutic range of an International Normalised ratio (INR) between 2.0-3.0, commonly expressed as the percentage time in therapeutic range (% TTR). Overall survival is improved for warfarin treated groups where TTR is greater than 40% [1] and a TTR of >58% is needed to be confident that patients will benefit from anticoagulation [2]. Patients with a TTR of ≥ 70% have a 79% reduced risk of stroke compared to those spending <30% of time in range, and have lower mortality rates [3]. Whilst unstable anticoagulation control has been associated with poor drug compliance, poor patient knowledge about anticoagulant treatment, acute or irregular alcohol use and genetics (*CYP2C9* and *VKORC1* polymorphisms), other factors including variability in and response to vitamin K intake, may contribute to poor stability [4].

In man, vitamin K is obtained primarily from the diet in the form of phylloquinones which are found in greatest concentration in green leafy vegetables [5], intake of which varies from day to day. The amount of dietary intake of vitamin K influences warfarin sensitivity and variability in INR [6]. Patients with poor dietary vitamin K intake are more susceptible to fluctuations in anticoagulation control [7], being more sensitive to day to day changes in vitamin K intake because of limited liver stores to stabilise the production of the functional clotting factors II, VII, IX and X, for which vitamin K is an essential co-factor [8]. Warfarin reduces the regeneration of vitamin K hydroquinone from vitamin K 2, 3-epoxide by inhibiting vitamin K epoxide reductase (VKOR) in the vitamin K cycle [9]. The VKOR enzyme is expressed by the vitamin K epoxide reductase gene, *VKORC1*, in which a number of common polymorphisms have been identified. These polymorphisms, which include a *G > A* polymorphism at *VKORC1* position *-1639* of Intron 1, are associated with altered hepatic VKOR expression and lower warfarin dose requirements [10, 11].

Stability of anticoagulation control can be improved by daily vi-

tamin K supplementation [6, 12], although an initial increase in warfarin dose may be required because of the antagonist effect of vitamin K on the pharmacological activity of warfarin. This varies between different patients and is related to *VKORC1-1639G > A* genotype, with those carrying the *GG* genotype demonstrating a significantly larger fall in INR, and requiring a significantly greater increase in warfarin dose, by an average of 33%, compared with 11% for those carrying the *GA*, and < 1% for those with the *AA* genotype, following vitamin K supplementation [13]. This is because patients with *GG* genotype are more efficient at converting vitamin K epoxide to vitamin K, leading to a greater regeneration of vitamin K hydroquinone and subsequently a larger increase in the activation of vitamin K dependent clotting factors compared to patients with the *GA* and *AA* genotype [13].

Based upon the above observation we hypothesized that patients on warfarin therapy with the *VKORC1(-1639) GG* polymorphism could have less stable control of anticoagulation than those with *GA* or *AA* genotypes as a result of variable dietary intake of vitamin K. We set out to examine this possibility in a cohort of patients on chronic warfarin therapy.

Materials and Methods

The INR values and dose changes of atrial fibrillation patients taking warfarin who had attended the Newcastle upon Tyne Anticoagulation Monitoring Service for at least 18 months for INR testing and dosage advice by staff guided by the DAWN dosing programme [14], and had *VKORC1–1639G > A* established in our previous studies of effect of *VKORC1* on warfarin dose requirements, were analysed. Since patients commencing warfarin require an initial dose titration period following a loading dose regimen to reach anticoagulation stability, the first six months from the time of warfarin commencement were excluded and the subsequent 12 months data analysed. TTR was calculated by the method of Rosendaal et al [15]. The study was approved by the Regional Ethics Committee and was in accordance with the Helsinki Declaration of 1975.

Statistical Analysis

Statistical analysis was conducted by Statistical Package for Social Sciences version 21 (SPSS). One way analysis of covariance (ANCOVA) was used to examine the difference in TTR, number of INR measurements, mean dose, and number of dose changes among the three *VKORC1* variants (*GG*/*GA*/*AA*) taking into account the effect of age and sex covariates.

Results

234 patients [106 (45%) female] were included in the study. The prevalence of *VKORC1 AA*, *GA*, and *GG* variants was 18.4%, 56.4%, and 25.2% respectively. The mean age ± SD between *AA*, *GA*, and *GG* variants groups were similar, 64 ± 18, 63 ± 15, and 63 ± 13, respectively. No statistical differences were observed between the three variants (*GG GA AA*) in mean TTR (66%, 61%, 68%, respectively), mean number of INR monitoring events (12.4, 13.5, 13.0, respectively), and mean dose changes (3.9, 4.0, 3.3, respectively). Similarly there were no significant differences in these measurements between *AA* and *GG* + *GA* genotypes. However, patients with *GG* variant required a significantly higher mean warfarin dose compared to those with *GA* variant (4.0 mg v 3.3 mg; P=0.02) with a mean dose difference of 0.7mg (p=0.02). However, no difference in dose requirement was observed between *AA* (mean dose=3.2 mg) and *GA* variants and between *AA* and *GG* variants (p=0.07). No influence of age and sex covariates was detected.

Discussion

Identifying and controlling factors which contribute to intra-individual variability in response to vitamin K antagonists would improve the average time patients stay within their therapeutic INR range, lowering the risk of bleeding or thromboembolic complications associated with over and under-anticoagulation, helping individual patients, especially the very elderly, who have difficulty attending for monitoring and receiving dosing instructions [16]. Polymorphisms in *CYP2C9* and *VKORC1* are relevant to this in clinical practice as both contribute to the inter-individual variability in coumarin dose requirement and stability of anticoagulation control during the initiation phase of therapy [4]. Excessive anticoagulation occurs more frequently in the carriers of *CYP2C9*3* variants (slow S-warfarin metabolisers) at the initiation of treatment and carriers have a lower TTR than those with the wild-type *CYP2C9* genotype [17, 18]. Compared to the *VKORC1-GG* genotype, the *GA* genotype results in less expression of *VKOR* enzyme, so that a lower dose of vitamin K antagonist is needed to achieve the same anticoagulant effect [17]. Our results, which show that *GG* genotype patients required a significantly higher mean warfarin dose compared to *GA* genotype patients, is consistent with this observation. Results of a study which employed a questionnaire assisted estimation to identify patients with low, medium and high vitamin K intake indicated that the influence of vitamin K intake on warfarin anticoagulation might be *VKORC1* genotype dependent as, in subjects with a variant *VKORC1 –1639G* allele, the mean daily dose of warfarin was significantly attenuated by low vitamin K intake compared with medium and high whilst no such effects were observed in homozygous patients for the *VKORC1-1639A* allele [13, 19]. We investigated the effect of *VKORC1* genotype on long-term control of anticoagulation in patients on chronic warfarin therapy, influenced by dietary vitamin K intake. Given that generally people enjoy having varied diets it is to be expected that there was sufficient variability in the patients' diet over the 12 months period their anticoagulation control was assessed. One limitation of the study is the lack of information on patients' comorbidities and concurrent therapy. However, these appear not to have made any significant contribution within the context of this study as the results revealed no difference in % TTR between the three *VKORC1* variants. It should be noted that the main objective of the current study was to investigate the long-term stability of anticoagulation control in association with *VKORC1* polymorphism with the background of a variable diet. Therefore polymorphisms in genes which either affect warfarin (*CYP2C9*) or vitamin K metabolism (*CYP4F2*) would have no effect on anticoagulation control in patients who are on chronic maintenance warfarin therapy.

Conclusion

Our results, which failed to confirm the hypothesis that long-term anticoagulation control is affected more in patients with the *VKORC1(-1639)GG* variant, in association with the expected variability in dietary vitamin K, indicate that the effect of any such influence at the pharmacological level is too small to influence clinically relevant outcomes in our population. However, it remains possible that a larger prospective study, in which dietary

intakes of vitamin K were measured, might yet establish a statistically significant link between diet, *VKORC1* polymorphism and anticoagulation control.

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