Thrombosis Research 171 (2018) 167–170

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Effect of genetic and patient factors on warfarin pharmacodynamics following warfarin withdrawal: Implications for patients undergoing surgery

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ARTICLE INFO

Keywords:
Warfarin
Anticoagulation
Thromboembolism
CYP2C9
VKORC1
Surgery

ABSTRACT

Introduction: Warfarin therapy is stopped for a fixed period prior to surgery to minimise risk of perioperative bleeding. However, anticoagulation subsides at varying rates among different patients. We evaluated the influence of genetic (*CYP2C9* and *VKORC1*), patient and clinical factors on warfarin clearance and the decline in INR following warfarin withdrawal.

Materials and methods: 131 patients completing a course of warfarin provided blood samples over 9 days for initial genotyping, and measurement of INR and plasma warfarin enantiomer concentrations.

Results: S-warfarin clearance was significantly lower in patients with either *CYP2C9* single (**2 or *3*) or double (**2*2 or *2*3*) variant alleles compared to those with wild-type genotype ($P < 0.001$). Regression analysis revealed that patient age ($P = 0.037$) and *CYP2C9* *2*2 & *2*3 genotype ($P = 0.005$), but not *VKORC1* genotype, significantly affected the time taken for the resumption of normal coagulation (INR value declining to ≤ 1.5).

Conclusions: The inter-individual variability in the time needed for normal coagulation to resume following warfarin withdrawal is influenced, in the main, by variance in S-warfarin clearance, which in turn is affected by CYP2C9 polymorphism and age. Cost-effectiveness of pharmacogenetics-based algorithms incorporating *CYP2C9* genotype and patient age could be increased if used not only to guide dosing decisions but also estimation of the correct length of time needed for individual patients to stop taking warfarin prior to surgery.

1. Introduction

Warfarin is prescribed as a 50:50 racemic mixture of (*R*)- and (*S*)-warfarin enantiomers, with the latter being 3–5 times pharmacologically more potent than the former. (*S*)-warfarin hydroxylation is influenced by polymorphisms in the *CYP2C9* gene; *CYP2C9*2* and *CYP2C9*3* variants encoding enzymes that are respectively around 12% and 5% as efficient as the wild-type (CYP2C9 *1*1) enzyme [1–3].

Vitamin K epoxide reductase, the pharmacological target enzyme for warfarin, recycles vitamin K epoxide to the reduced active form of vitamin K (vitamin K hydroquinone), an important cofactor needed in the functionalisation of clotting factors II, VII, IX, and X by γ–glutamyl carboxylation of their N-terminals. The enzyme is encoded by the vitamin K epoxide reductase complex subunit 1 gene (*VKORC1*); polymorphisms in this gene have been identified and linked with clotting factor deficiencies and warfarin resistance [4].

Although the contribution of *CYP2C9* and *VKORC1* genotypes to warfarin dose requirements is well established [5–7], the extent of their influence upon the recognised inter individual variation in the rate of INR decay after cessation of warfarin therapy in patients awaiting surgery is not. An earlier study indicated that sensitivity to warfarin, advanced age and extreme elevation of the INR are independent risk factors for prolonged delay in return of a supra-therapeutic INR to the target range [8]. In patients following established recommendations to stop their warfarin 5 days prior to an invasive procedure [9], rate of INR decline varies, with 23% having an INR > 1.2 after 4.7 days of withdrawal from warfarin in one study [10], 7% having a pre-operative INR > 1.5 after five days with subsequent need for vitamin K administration prior to surgery in another [11]. In our previous study of the effect of *CYP2C9* genotype upon INR decline, patients with two *CYP2C9* variant alleles (*CYP2C9*2*2* or *CYP2C9*2*3*) were found to be over 8 times more likely (95% [12] = 2.25–33.25) to have an INR of ≥1.5 on the day before planned surgery, compared to those with either **1*1*, **1*2* or **1*3* genotypes. The possible impact of *VKORC1* polymorphism

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https://doi.org/10.1016/j.thromres.2018.09.064
Received 17 July 2018; Received in revised form 27 September 2018; Accepted 28 September 2018
Available online 01 October 2018

on the fall in INR was not evaluated [13]. The aim of the current study was to characterise further the contribution of genetic, patient and clinical factors toward warfarin elimination and its changing pharmacodynamics in patients with thromboembolic disease following warfarin withdrawal and to determine the potential clinical utility of a personalized pharmacogenetics-based approach in guiding management of patients on warfarin scheduled for invasive surgery.

2. Methods

The study had the approval of the Newcastle upon Tyne Ethics Committee. All patients provided written informed consent according to the Declaration of Helsinki. One hundred and thirty five Caucasian patients aged over 18 years, completing a course of warfarin for indications of either venous thromboembolism (VTE), or atrial fibrillation (warfarin therapy stopped after successful cardioversion), with a target INR of 2.0–3.0 and stable control of anticoagulation (defined as having a stable warfarin dose for at least the previous 2 clinic visits a minimum of one week apart), were identified through the anticoagulant clinic records at the Newcastle upon Tyne Hospitals NHS Foundation Trust, UK. Any patient with a condition which might affect warfarin metabolism, notably congestive cardiac failure, hepatobiliary or renal disease or active cancer, taking medication known to interact with warfarin, or drinking more than the UK recommended safe limits (14 units per week for men and women) [14] was excluded.

On the day of warfarin withdrawal, demographic data of age, weight, sex, and indication for anticoagulation therapy, medical diagnoses, daily warfarin dose, concomitant medication and alcohol intake were recorded. A blood sample (10 ml) was taken for measurement of baseline INR and *CYP2C9* and *VKORC1* genotyping. Thereafter, each patient attended the clinic on 3–4 separate occasions, spread over 9 days, beginning the day after their last dose of warfarin, with some variation to avoid scheduling conflicts (e.g. weekends) and missed appointments. A venous blood sample (10 ml) was collected at visit one (16–20 h after the last dose of warfarin) for measurement of steady-state plasma warfarin enantiomer concentrations and INR. A venous blood sample (10 ml) was taken at each of the remaining study visits for INR measurement only.

Plasma warfarin enantiomer concentrations were determined by high performance liquid chromatography (HPLC) using the method of Naidong, et al. [15]. The limit of detection of the extracted samples was 2 ng/ml (signal/noise ratio = 3). The inter-day coefficient of variation for S- and R- warfarin at 690 ng/ml was 4.2% and 3.8%, respectively. *CYP2C9* and *VKORC1–1639* genotyping were carried out according to an established method [5]. The rate of decline in INR for individual patients was determined from the slope of the plot of INR versus time. S- and R- warfarin clearance (CL) was calculated according to the formula: D/(2 t X Css.av), where D is the daily dose of warfarin (mg), t is the dosing interval (24 h), and Css.av. is the average steady-state plasma warfarin enantiomer concentration (mg/L), assuming that warfarin compliance and bioavailability are 100% [16].

2.1. Sample size calculation

According to previous studies INR reached a value of < 1.5 in 74 ± 19 h following warfarin withdrawal [10,11]. To achieve 80% power and to detect a 15 h difference in the time needed to reach an INR < 1.5 between individuals with the *CYP2C9 *1*1* genotype and either those with at least one variant allele (i.e. either *CYP2C9*2* or *CYP2C9*3*) and allowing for the expected proportion in the three genotype groups, a sample size of 135 patients were needed to be studied.

2.2. Statistical analysis

Statistical analysis of the data was carried out using Minitab software version 17 (Coventry, UK). The effects of study variables on INR were assessed using multiple and logistic regression models. By regressing the logarithm of INR against time for each individual, the time for INR to reach 1.5 was estimated. This estimated time was modelled using *CYP2C9* and *VKORC1* genotypes, and other study variables. When necessary, data were transformed into their logarithmic values to approach normality. One-way analysis of variance (ANOVA) was used for comparison of continuous data. A $P < 0.05$ was taken as being statistically significant. Data are presented as mean ± SD unless otherwise stated.

3. Results

One hundred and thirty five patients were recruited into the study. However, for statistical analysis data were available for 131 participants [73 (56%) males] as four later withdrew from the study due to inability to attend study visits. The age range for the study cohort was 21–92 years; there was no significant difference in mean age between males and females (62 ± 15 versus 64 ± 18 years). At the point of recruitment, patients were taking a median of 2 drugs (IQR 1–5), excluding warfarin, 1 of which affected the cardiovascular system. Of drugs reported as potential inhibitors of CYP2C9, 1 patient was taking clopidogrel and 1 phenytoin, a drug which is also reported as a potential inducer of CYP2C9. Two patients were taking the potential inducer, carbamazepine. Forty one patients were taking statins of whom 30 were taking simvastatin, 9 atorvastatin, 1 rosuvastatin and 1 pravastatin. No patient was taking amiodarone or fluvastatin. As well as indications for warfarin (atrial fibrillation 11%, deep venous thrombosis 61%, pulmonary embolism 26%, and valvular prosthesis 2%), the median number of comorbidities was 1 (IQR 1–2), most commonly cardiovascular disease (69%). The frequency of *CYP2C9* and *VKORC1* genotypes in the study population are presented in Table 1.

On the screening day following the final warfarin dose (warfarin dose usually taken early evening the day before), mean INR was 2.4 ± 0.6. At the first study visit (16–20 h after the last warfarin dose), the proportion of INR values ≥1.5 among those with *CYP2C9* wild-type, single variant, and double variant genotype was 78%, 94%, and 100%, respectively; *CYP2C9* genotype was the only predictor of an INR < 1.5 ($P = 0.009$) at the first visit; the odds ratio for the single variant genotype relative to the wild-type was 0.22 (95%CI = 0.06, 0.79). The proportion of INR values ≥1.5 at the second visit (mean of 102 h) among those with *CYP2C9* wild-type, single- and double-variant alleles was 9%, 15%, and 40%, respectively, but these differences were no longer statistically significant; no further genetic influences were identifiable beyond the second visit as most of the INR values were close to 1.0 by the third and fourth study visits.

According to regression analysis, which included the variables of *VKORC1* and *CYP2C9* genotype, age, weight, sex, warfarin dose,

Table 1
CYP2C9 and *VKORC1* genotype frequency distribution in the study population.

CYP2C9 polymorphism, n (%)	
**1*1*	72 (56.7)
**1*2*	33 (26.0)
**1*3*	15 (11.8)
**2*2*	6 (4.7)
**2*3*	1 (0.8)
VKORC1 polymorphism, (%)	
GG	49 (38.6)
AG	63 (49.6)
AA	15 (11.8)

N.B. Genotyping data missing for 4 patients.