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Genotype-driven pharmacokinetic simulations of warfarin levels in Puerto Ricans

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Abstract

Objectives: The inter-individual variability of warfarin dosing has been linked to genetic polymorphisms. This study was aimed at performing genotype-driven pharmacokinetic (PK) simulations to predict warfarin levels in Puerto Ricans.
Methods: Analysis of each individual dataset was performed by one-compartmental modeling using WinNonlin®v6.4. The k_e of warfarin given a cytochrome P450 2C9 (*CYP2C9*) genotype ranged from 0.0189 to 0.0075 h^{-1}. K_a and V_d parameters were taken from literature. Data from 128 subjects were divided into two groups (i.e., wild-types and carriers) and statistical analyses of PK parameters were performed by unpaired t-tests.
Results: In the carrier group (n=64), 53 subjects were single-carriers and 11 double-carriers (i.e., *2/*2, *2/*3, *2/*5, *3/*5, and *3/*8). The mean peak concentration (Cmax) was higher for wild-type (0.36±0.12 vs. 0.32±0.14 mg/L). Likewise, the average clearance (CL) parameter was faster among non-carriers (0.22±0.03 vs. 0.17±0.05 L/h; p=0.0001), with also lower area under the curve (AUC) when compared to carriers (20.43±6.97 vs. 24.78±11.26 h mg/L; p=0.025). Statistical analysis revealed a significant difference between groups with regard to AUC and CL, but not for Cmax. This can be explained by the variation of k_e across different genotypes.
Conclusions: The results provided useful information for warfarin dosing predictions that take into consideration important individual PK and genotyping data.

Keywords: *CYP2C9* polymorphisms; genotype; pharmacokinetics; Puerto Ricans; simulations; warfarin.

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Introduction

Warfarin is one of the most widely prescribed oral anticoagulant drugs approved by the Food and Drug Administration (FDA) for the treatment and prevention of thromboembolic events (Supplementary Material, Figure S1) [1]. It is also on the World Health Organization (WHO) Model List of Essential Medicines. Traditionally, warfarin therapy is affected by the patient's concurrent medication use, age, body weight, and liver function [2]. Warfarin's effectiveness is contrasted by its required close monitoring due to a narrow therapeutic window, interaction with multiple drugs and foods, individual-to-individual response variability and the increased risk of fatal bleeding. The IMS Health National Prescription Audit has previously included warfarin among the top 25 prescription medicines dispensed in the United States [3]. In the past, warfarin has ranked second on the FDA rankings of drug adverse event cases reported with 1,106 cases overall, including 72 deaths [4]. Warfarin is essentially completely absorbed after oral administration and has an apparent volume of distribution of about 10 L/70 kg

(0.14 L/kg). For most purposes a one compartment model is used to describe the disposition of warfarin after oral dosing [5]. Hamberg et al. described the disposition of S-warfarin as a one-compartment model with first-order input and first-order elimination [6]. With this type of model and assuming complete bioavailability, the volumes of distribution of the R- and S-enantiomers are similar to each other and to that of the racemate [5, 6].

The variability in response to warfarin among individuals has been linked in the literature to the genetic variation of mainly two pharmacogenes (i.e., *CYP2C9* and *VKORC1*) [2]. The anticoagulant potency of S-warfarin is much greater than that of R-warfarin. Metabolism of S-warfarin in the liver is mainly mediated by the cytochrome P450 2C9 (CYP2C9) drug-metabolizing enzyme, a major pathway in humans that converts this drug into its inactive hydroxylated metabolites [2]. Accordingly, polymorphisms on the *CYP2C9* gene contribute to the variability in response to warfarin therapy [2]. The other gene involved is the one encoding for the molecular target of warfarin, named vitamin K epoxide reductase complex subunit 1 (*VKORC1*) [2, 7]. In 2007 and 2010, the FDA approved updates on warfarin prescribing information twice to emphasize the opportunity for healthcare providers to use genetic test results to predict effective warfarin dosing based on individual genetic information [8]. This allowed the use of pharmacogenomics and related prediction algorithms to optimize the warfarin dose estimation and reduce the risk of bleeding complications associated to warfarin [7–9].

If patients' genotypes predict the changes in the elimination rate constant of warfarin, then we could use this information to predict warfarin pharmacokinetics (PK) differences between wild-types and carriers using WinNonlin® simulations. The aims of this study are to predict individual warfarin PK profiles and related parameters as well as to compare the estimated PK parameters between non-carriers (wild-types) and carriers of *CYP2C9* polymorphisms. To this purpose we performed WinNonlin® simulations of warfarin PK profiles using previously collected genotyping data of different *CYP2C9* polymorphisms present in a cohort of Puerto Rican patients. These genotype-driven PK simulations of warfarin serum levels and parameters are expected to expand our current understanding of and fill a knowledge gap on the clinical utility of pharmacogenetics to explain the observed inter-individual variability in warfarin response among Puerto Ricans. To our knowledge a genotype-driven PK simulation to predict warfarin serum levels in the Puerto Rican population has not been previously performed. The ability to predict warfarin levels using a PK model will provide useful data in order to establish a relationship between existing genetic polymorphisms and resulting serum levels.

Materials and methods

This is a secondary analysis of a previous pharmacogenetic study of warfarin in Puerto Rican patients (IRB approval #A4070109). Proper safeguards against any potential violation of privacy and/or breach of confidentiality will be ensured. Authorization to use the data for the purpose stated in this project was previously obtained from individual patients by an informed consent process. Accordingly, the study was conducted following the Helsinki's declaration for human subject protection in clinical surveys. Participants were mostly males and elderly patients receiving warfarin for different thromboembolic disorders at the anticoagulation clinic in the Veteran Affairs Caribbean Healthcare System (VACHS) at San Juan, PR. The inclusion and exclusion criteria for the enrollment of subjects were the same as in the parent study (Supplementary Material, Table S1), where further details can be found [10, 11].

Study design

Based on literature reports [5], we selected an oral one-compartment, mammillary open model to predict individual plasma warfarin concentrations (response variable) by simulation of both single and multiple-dosing schemes through WinNonlin® software (version 6.3, Pharsight Co.). The equations of the models to predict plasma warfarin concentrations (Cp) at any time (t) are as follows [12, 13]:

Single dose:

$$Cp = \frac{k_a F D_0}{V_d (k_a - k_e)} e^{-k_e t}$$

Multiple dose regimens:

$$Cp = \frac{k_a F D_0}{V_d (k_a - k_e)} \left[\left(\frac{1}{1 - e^{-k_e \tau}} \right) e^{-k_e t} - \left(\frac{1}{1 - e^{-k_a \tau}} \right) e^{-k_a t} \right]$$

where, D_0 is the maintenance effective dose per day in milligrams (mg) according to the information available in the computerized patient record system (CPRS) (electronic patient's medical record at VACHS); k_a is the fixed first order absorption rate constant (1.19 h^{-1}) [14]; F is the oral bioavailability of warfarin according to literature (100%) [5]; V_d is the population average volume of distribution of warfarin according to literature (0.14 L per kilogram of body weight) [5]; t is the time elapsed after the dose was administered; and k_e is the elimination rate constant of warfarin given a *CYP2C9* genotype: *1/*1=0.0189 h^{-1} (wild type); *1/*2=0.0158 h^{-1}; *1/*n=0.0132 h^{-1}; *2/*2=0.0130 h^{-1}; *2/*n=0.009 h^{-1}; *n/*n=0.0075 h^{-1}, being n=*3, *5, *6 or *8 [15]. For multiple-dose regimens, a dosing interval (τ) of 24 h was used. Multiple-dose regimens refer to the daily administration of warfarin used in treatment in order to maintain the plasma drug levels within the narrow limits of the therapeutic window and to achieve optimal clinical effectiveness [13].

Sample size

Based on previous reports, when a one-compartment PK modeling analysis is performed, a minimum sample size of 30 subjects is

required in order to achieve a 95% confidence interval and a power of 0.9 [16]. All subjects carrying a *CYP2C9* polymorphism (n=64) were selected out of the 260 patients with complete genetic and clinical data from the VACHS study cohort. An equal number of non-carrier subjects were randomly selected for a total "balanced" sample size of 128 subjects.

Study variables

The independent variables were individual genotypes; individual volume of distribution, time elapsed from the initial dose, and maintenance effective dosing data retrieved from an available database (protocol# A4070109) and the CPRS at VACHS. For the purpose of this study, maintenance effective dose is defined as the stable warfarin dose where three consecutive International Normalized Ratio (INR) measurements were obtained within the expected target therapeutic values (2–3 or 2.5–3.5) for the same average weekly dose. The response variable is the individual plasma warfarin concentration (Cp) at any time (t) to be predicted by WinNonlin® simulations.

Data analysis

In the corresponding PK data analyses, datasets of every individual patient were used (Supplementary Material, Tables S2). One advantage of PK simulations is that it no longer depends on measurement of S-warfarin concentrations; this increases its applicability given that data relating to warfarin plasma levels are rarely available [6]. A PK simulation analysis of each individual dataset was performed by one-compartmental analysis using a modified Levenberg-Marquardt algorithm. A time zero value was considered for extrapolation purposes. Parameters were computed with their corresponding variance estimates and extrapolated to infinity (i.e., model-predicted). Computing these parameters based on the last observed level was discouraged in order to avoid larger estimation errors. Time to peak values were also determined as the time in which the maximum level was observed (i.e., maximum plasma concentration) considering the entire curve; and peak level was that corresponding to the above mentioned time to peak value. The maximum concentration to area under the curve ratio (Cmax/AUC) was also computed as an indicator of the extent of bioavailability. For all these purposes the WinNonlin® professional software (Version 6.3, Pharsight, Inc./Certara, 2014, NC, USA) was used.

Statistical analysis

The results were divided into two groups (i.e., non-carriers and carriers) and descriptive statistics were used to compare the PK parameters. Inferential statistics were used to compare the volume of distribution (V_d), area under the curve (AUC), maximum concentration (Cmax), and clearance (CL) between the carrier and non-carrier groups. Normality tests for each of these parameters were performed using the Kolmogorov–Smirnov test and a t-test with a significance of 0.05 was used for the comparison. For parameters that failed the normality test, the data was normalized by logarithmic transformation using the natural logarithm function (Ln). All of the statistical tests were done using Minitab 17 Statistical Software®.

Results

All of the 128 subjects studied were males and, of these, 64 carried genetic polymorphisms (carriers) and 64 did not (non-carriers). In the carrier group, two subjects carried the *3/*5,*8 polymorphism, seven subjects carried the *2/*3,*5 polymorphism, two subjects carried the *2/*2 polymorphism, 23 subjects carried the *1/*3,*5,*8 polymorphism and 30 subjects carried the *1/*2 polymorphism. The non-carrier group had an average weight of 83.6±11.8 kg with an average V_d of 11.7±1.6 L, while the carrier group had an average weight of 87.1±16.4 kg with an average V_d of 12.2±2.3 L. Results of PK simulations and statistical comparisons are presented in Figure 1A–E, Table 1 and Supplementary Material, Tables S3, S4. The genotype carrier group had a larger average time to peak value than non-carrier subjects (3.54±0.00 h vs. 3.81±0.16 h), whereas the average Cmax was higher for the non-carrier subjects than for the genotype carrier subjects (0.36±0.12 mg/L vs. 0.32±0.14 mg/L). The average CL was greater for the non-carrier group (0.22±0.03 L/h) than for the carrier group (0.17±0.05 L/h). The genotype carrier group showed a higher average AUC when compared to the non-carrier group (24.78±11.26 h*mg/L vs. 20.43±6.97 h*mg/L). Two-sample t-tests comparing the PK parameters of both groups showed a significant difference between the mean AUC [95% CI (–0.3029, –0.0202), p-value=0.025], mean $t½$ [95% CI (0.04827, 0.07611), p-value=0.044] and the mean CL [95% CI (0.03872, 0.06621), p-value<0.0001], but no significant difference between the mean V_d [95% CI (–0.0907, 0.0219), p-value=0.229] and the mean Cmax [95% CI (–0.0013, 0.0902) p-value=0.057].

Discussion

Polymorphisms in both the *VKORC1* and *CYP2C9* loci combined have been postulated to explain in part the observed variability in warfarin dose requirements [2, 7, 9]. Indeed, a growing body of evidence has emerged indicating that *CYP2C9* and *VKORC1* genotypes are associated with maintenance dose requirements and account for up to 40–45% of the inter-individual variability, depending on the population and specific polymorphisms studied [18]. Some patients with genetic variants of reduced function in *CYP2C9* require a lower dose of warfarin and a longer time to reach a stable dose [9]. Given the obvious impact of *CYP2C9* and *VKORC1* polymorphisms on warfarin dose requirements, the FDA updated the warfarin dosing label in 2007 and 2010 [8, 17]. Valentin et al. described the association of *VKORC1* and

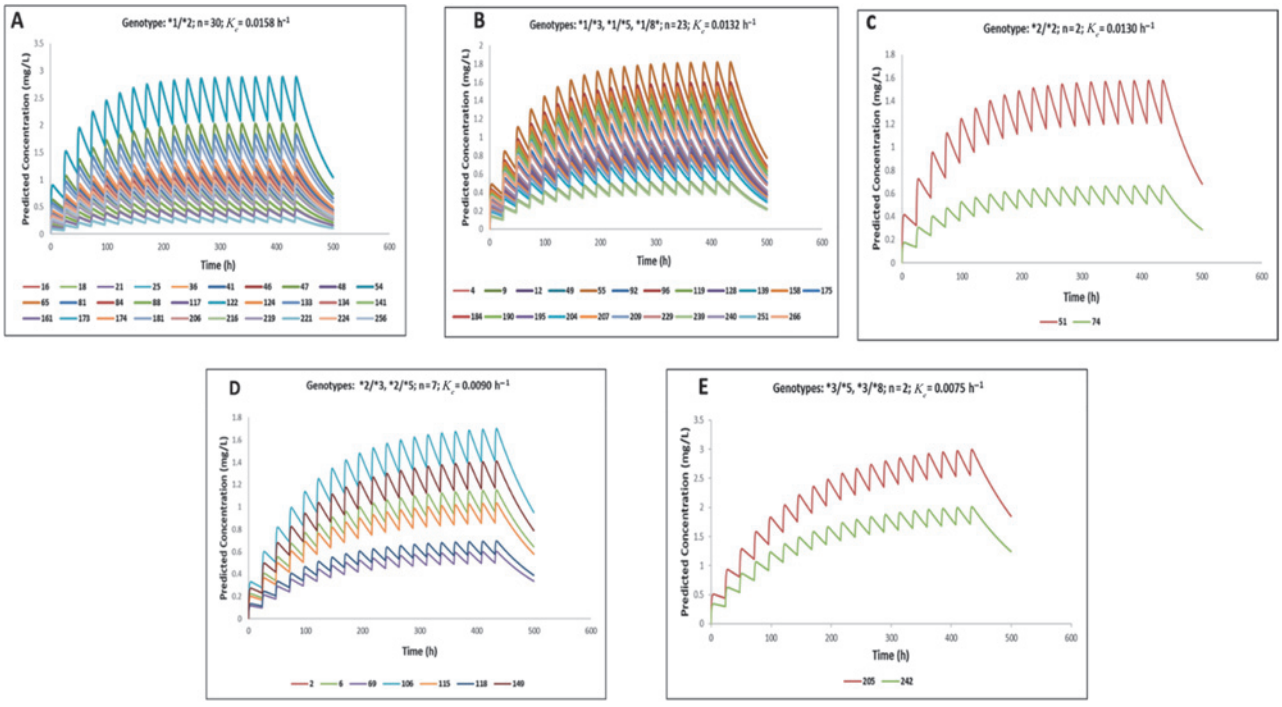

Figure 1: Simulated PK time profiles predicted concentration of S-Warfarin.
(A) S-warfarin concentration-time profile for individuals con genotype*1/*2 and K_e 0.0158 h^{-1}. (B) S-warfarin concentration-time profile for individuals con genotype*1/*3,*1/*5, *1/*8 and K_e 0.0132 h^{-1}. (C) S-warfarin concentration-time profile for individuals con genotype*2/*2 and K_e 0.0130 h^{-1}. (D) S-warfarin concentration-time profile for individuals con genotype*2/*3,*2/*5 and K_e 0.0090 h^{-1}. (E) S-warfarin concentration-time profile for individuals con genotype*3/*5, *3/*8 and K_e 0.0075 h^{-1}.

Table 1: Statistical comparisons of relevant PK parameters between carriers and non-carriers. Two-sample unpaired t-tests were performed (5% significant level).

PK parameters	Non-carriers	Carriers	p-Value
Volume of distribution (V_d, L)	11.7 ± 1.6	12.2 ± 2.3	0.229
Elimination half-lives ($t½$, h)	3.54 ± 0.01	3.81 ± 0.16	0.044[a]
Peak drug concentrations (Cmáx, mg/L)	0.36 ± 0.12	0.32 ± 0.14	0.057
Clearance (CL, L/h)	0.22 ± 0.03	0.17 ± 0.05	0.0001[a]
Area under curve (AUC, h*mg/L)	24.78 ± 11.26	20.43 ± 6.97	0.025[a]

[a]Means statistically significant difference.

CYP2C9 genotypes to effective warfarin doses in Puerto Ricans and used pre-existing algorithms to predict warfarin initial dose requirements [10]. They showed that patients with one or two polymorphisms had reduced warfarin metabolism, thus requiring lower initial doses. The predicted dose reductions ranged from 1.6 mg/day–4.9 mg/day [10].

Kaye et al. indicated genotype-guided warfarin dosing algorithms are a rational approach to optimize warfarin dosing and potentially reduce adverse drug events [17]. More genetically diverse populations such as African Americans and Hispanics or Latinos have greater variability in warfarin dose and higher risk for warfarin-related adverse events compared to individuals of European ancestry, which suggest that they may benefit more from improving warfarin dose estimation using pharmacogenomics. Overall, some genotype-guided algorithms developed in Puerto Ricans have predicted warfarin doses more accurately [10, 11], which demonstrate to some extent the utility of genotype-guided warfarin dosing. Likewise, a Puerto Rican-specific pharmacogenetic-based warfarin dosing algorithm to predict individual initial doses for patients commencing anticoagulation therapy was developed and explained about 48% of observed variability. In this study, a pharmacokinetic and pharmacodynamic (PK-PD) analysis was also conducted [15].

The data obtained from the WinNonlin simulations were used to describe differences in PK parameters between Puerto Rican subjects with and without *CYP2C9* genetic polymorphisms. The characterized data revealed PK profiles in accordance with their respective genotypes. It was also observed that individual values of the parameter k_e affected the CL (as expected) and the AUC of each PK profile, but did not have any effect on V_d values and Cmax.

The results of this study showed that the mean V_d value (L) was larger in the carriers group than that for non-carriers. This observation is in agreement with the average body weight (BW, kg) reported by patients in the carriers group, who showed higher body weights than those reported within non-carriers. Accordingly, the V_d values were on average higher for carriers due to larger body weights in this group. However, statistical analysis showed no significant difference between mean V_ds in both study groups (11.7±1.6 vs. 12.2±2.3 L; p-value of 0.229). Also, the average peak level (Cmax, mg/L) was higher in the group of non-carriers even though the corresponding average AUC (h mg/L) was lower and the average CL (L/h) was higher in this group. These results can be explained by the fact that non-carriers would need higher warfarin maintenance doses to reach the therapeutic INR range. The carriers are slow or poor metabolizers and, therefore, need lower maintenance doses to reach the target INR.

Statistical analyses revealed significant differences between both the mean AUCs (24.78±11.26 vs. 20.43±6.97 h mg/L; p-value of 0.025) and mean CLs (0.22±0.03 vs. 0.17±0.05 L/h; p-value of 0.0001) between patients from both groups, which is explained by the relationship between the k_e and these two parameters. The higher the k_e the faster the CL, and consequently, the lower the AUC. Since k_e depends on *CYP2C9* genotypes (i.e., being higher for non-carriers and then slowing down among patients who are carriers of a clinically relevant *CYP2C9* variant, with an effect size that depends ultimately on the impact of each genetic polymorphism on the enzyme activity), it is expected that larger effect are observed among those in the carrier group. From these results, it can also be inferred that the CL parameter was affected mainly by the variations in k_e as a result of the *CYP2C9* polymorphism.

The statistical analysis also revealed no significant differences between both groups with regard to their Cmax values (0.36±0.12 vs. 0.32±0.14; p-value of 0.057). Since all 128 subjects were taking their corresponding maintenance effective ("stable") warfarin doses at the moment of genotyping, we might speculate that this finding of no significant differences in peak levels among patients result from the adoption of optimal strategies for warfarin dosing adjustments over time in each individual. Important to bear in mind that a maintenance effective ("stable") dose of warfarin, which is part of the inclusion criteria, is defined when the following condition is met; i.e., three consecutive INR measurements within the expected therapeutic range for the same average weekly dose. Consequently, if each maintenance effective ("stable") dose of warfarin was individualized to get their respective INR values on the target range, it is fairly reasonable to expect that the corresponding warfarin concentrations should also be within range for everyone.

The relevance of this finding is that current warfarin dosing prediction algorithms do not take into account the plasma levels and most ongoing studies are focused on the creation of pharmacogenetic models without taking into account PK parameters. Further efforts are needed to increase the use of available warfarin PK data in order to perform sound analysis of the potential impact of patient's genetics. Although still limited, there are a few other studies in non-Hispanics populations that have also used PK models to predict the effect of *CYP2C9* polymorphisms on warfarin dosing [6, 19, 20]. Overall, these studies have concluded that PK may contribute to >10–20-fold inter-patient variability in dose requirements, which confirm in part our findings in Puerto Ricans. The PK models for warfarin in such studies had V_{ds} of 15.2±3.2 L and CLs ranging from 0.088–0.174 L/h [6, 19]. These values compare well with the corresponding estimates of such parameters for patients in our study (i.e., V_{ds} from 11.7±1.6 [non-carriers]–12.2±2.3 [carriers] L; and CLs from 0.17±0.05 [carriers]–0.22±0.03 [non-carriers] L/h). On the other hand, a fixed k_a of 1.66 h^{-1} was used in the study by Lane et al. [19]; whereas, Hamberg et al. [6] used a fixed k_a value of 2 h^{-1}. Other values, ranging from 1–5 h^{-1}, have also been tested in sensitivity analyses with no impact on the model results [19]. A fixed k_a value of 1.19 h^{-1} was used for our PK simulations, which is within the above range. Future studies are also warranted to assess the relationship between warfarin serum concentrations, effective doses, therapeutic INRs and the presence of a genetic polymorphism. Measured or predicted warfarin concentrations in blood may be of importance in increasing the prediction power of future dosing algorithms in Puerto Ricans.

Our study has some limitations. First, this was a single-center study with a retrospective study design, which can increase chances for missing or even overlooking some attributes of the population. Second, the population included in this study was mostly elderly and all of the evaluated subjects were male, which fails to take into account possible effects of gender and age on PK parameters. Third, larger samples of each specific polymorphism are needed in order to validate our results. Finally, and perhaps the major limitation of this study, is the lack of determination of true plasma warfarin concentrations. However, we firmly believe these genotype-driven PK simulations of warfarin levels and parameters helped us expand our current understanding of the influence of pharmacogenetics on the observed inter-individual variability in warfarin dosing among Puerto Ricans.

Conclusions

The results obtained from our WinNonlin simulations provide useful information that can encourage the development of warfarin dosing algorithms by taking into consideration important PK data. Differences in genetic variations in both CYP and vitamin K-related genes could explain the diversity in warfarin sensitivity and dose requirement. The impact of genetic variants on warfarin dosage requirement might vary across different population worldwide. Together with non-genetic predictors the genetic variants might be used to improve warfarin dose prediction [21, 22]. In this study we were able to learn about the impact of *CYP2C9* genotypes on the individualization of warfarin therapy in Puerto Ricans from a PK perspective. In so doing, we provided further evidence on the influence of *CYP2C9* polymorphisms on the observed variability in stable warfarin dose among patients from our study cohort. Further research is needed to validate the findings of this study and subsequent studies should aim to increase the number of subjects that carry the various *CYP2C9* polymorphisms. Finally, we firmly believe this work contributes to the field by assessing the role of *CYP2C9* pharmacogenetics on inter-patient variability of predicted plasma warfarin concentrations and related PK parameters, emphasizing on the importance of interpreting genotyping results in the context of their PK and PD consequences.

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