Clinical verification of Lou type warfarin pharmacokinetic dosing algorithms equation

JIANGANG JIANG[1], NINGNING JI[2], JINGLIANG LAN[1], XIAOPING GE[3] and XIAOMA DU[1]

[1]Department of Cardiology, Jinhua Hospital of TCM Affiliated to Zhejiang University of Traditional Chinese Medicine, Jinhua, Zhejiang 321000; [2]Department of Cardiology, Yiwu Central Hospital, Yiwu, Zhejiang 322000; [3]Department of Geriatrics, Zhejiang Jinhua Guangfu Hospital, Jinhua, Zhejiang 321000, P.R. China

Received December 23, 2016; Accepted December 22, 2017

DOI: 10.3892/mmr.2018.8562

Abstract. Warfarin is the most commonly used oral anti-coagulant in clinic practice. However, it is difficult to recommend the correct dosage due to its narrow therapeutic window. The aim of the present study was to verify the clinical value of the Lou type equation, using pharmacogenetics-based warfarin dosing algorithms to appropriately predict the actual maintenance dose. A total of 87 Chinese Han patients who required treatment with warfarin were enrolled and randomly divided into the experimental and control groups. In the experimental group, the first 3 doses of warfarin were calculated according to the Lou type equation. While in the control group, these 3 treatments were performed following the doctors' recommendations. Then the dose of warfarin was gradually adjusted to the stable dose according to the changes in the international standardized ratio. At the end of the 50 day experimental period, there were a greater number of patients in the experimental group who exhibited a stable blood concentration of warfarin than those in the control group (83.35 and 64.4%, respectively). In addition, the mean and median times for patients to obtain a stable dose in the experimental group were significantly shorter than those in the control group (mean, 18.2±1.7 and 27.3±2.0 days; and median, 11.7±1.1 and 20.5±1.8 days, respectively). The adverse reaction rate of the experimental group (9.5%) was markedly lower than that of the control group (26.7%). The occurrence of adverse reactions in the experimental group was also significantly later when compared with the control group (43.9±1.6 and 38.6±1.5 days, respectively). Furthermore, there was no significant difference between the average predicted dose (3.4±1.1 mg/day) and the average actual dose (3.5±1.4 mg/day; P=0.313). In conclusion, using the Lou type warfarin pharmacokinetic dosing algorithm equation to administer warfarin markedly shortened the adjustment time of warfarin to reach a stable dose and reduced the adverse reactions rate, thus supporting clinical feasibility.

Correspondence to: Dr Jiangang Jiang, Department of Cardiology, Jinhua Hospital of TCM Affiliated to Zhejiang University of Traditional Chinese Medicine, 439 Shuangxi West Road, Wucheng, Jinhua, Zhejiang 321000, P.R. China
E-mail: jjg2009768@163.com

Key words: warfarin, Lou type equation, dose prediction algorithm, pharmacogenetics, gene polymorphism

Introduction

Warfarin is the most commonly used oral anti-coagulant in clinical practice and is widely used in the prevention and treatment of various types of deep venous thrombosis, pulmonary embolism, prosthetic heart valves and atrial fibrillation. Warfarin is characterized by its narrow therapeutic window, which means that a small change in concentration can cause serious side effects (1,2). Due to differences in the metabolism of warfarin in individuals, inadequate or excessive use of warfarin can lead to thrombus or hemorrhage (1,2). Genetic and non-genetic factors, including age, sex, race, weight and drug interactions can account for the better patient responses to specific doses (2-5). So the genetic polymorphisms contribute to the individual differences in warfarin dose response (3-5). A number of clinical research studies have revealed that a predictive equation based on pharmacogenomics is reliable for determining the dosage that is affected by individuals' genetic characteristics (6-11). Genetic polymorphisms of cytochrome P450 family 2 subfamily C member 9 (*CYP2C9*), vitamin K epoxide reductase complex subunit 1 (*VKORC1*) and cytochrome P450 family 4 subfamily F member 2 (*CYP4F2*) may result in 30-50% of individual warfarin dose variability (12-14). Lane *et al* (15) reported that *CYP2C19* and *CYP3A4* genotypes had a profound effect on R-warfarin clearance. Rieder *et al* (16) found that the γ-glutamyl carboxylase (*GGCX*)-12970 SNP was associated with the warfarin maintenance dose. Chung *et al* (17) confirmed that polymorphisms of microsomal epoxide hydrolase 1 (EPHX1) and VKORC1-like 1 (VKORC1L1) could contribute to the warfarin dose variability. A number of studies and clinical trials regarding the pharmacogenomics of warfarin have been conducted worldwide, and they have yielded a number of different drug equations (4,5,10,18,19). However, the value of each equation in the clinical application between different races in different regions is quite different (3-5,7,13). A study published by Lou *et al* (5) in May 2014 identified the stable dose of warfarin by evaluating Han Chinese patients and the

results were of particular interest. The Lou type warfarin pharmacokinetic dosing algorithm equation was based on the genetic polymorphisms of CYP2C9, VKORC1, and CYP4F2 and other non-genetic variables. In the present study, the Lou type warfarin pharmacokinetic dosing algorithm equation was applied to verify the efficacy of warfarin in clinical treatments via a randomized, controlled prospective study of Han Chinese patients in Zhejiang, which supported the implementation of medicine stemming from clinical studies.

Materials and methods

Patients. The present study was approved by the Ethics Committee of the Jinhua Hospital of TCM Affiliated to Zhejiang University of Traditional Chinese Medicine (Zhejiang, China). Patients (n=87; Table I) who were admitted to Jinhua Hospital of TCM Affiliated to Zhejiang University of Traditional Chinese Medicine (Zhejiang, China), Yiwu Central Hospital (Zhejiang, China), and Zhejiang Jinhua Guangfu Hospital (Zhejiang, China) and Rehabilitation Hospital of Yiwu (Zhejiang, China) and required warfarin treatment were recruited to the present study between June 2014 to 2016. Written informed consent was obtained from all participants.

Inclusion and exclusion criteria. The inclusion criteria were as follows: i) Patients were >18 years and were able to behave independently; ii) patients with sufficient medical data available; and iii) patients consented to a 50 day experimental period following treatment. The exclusion criteria were as follows: i) Patients with no serious bleeding within the last 6 months; ii) patients who rejected long-term anti-coagulant therapy; and iii) patients were unable to complete the study.

Lou type warfarin pharmacokinetic dosing algorithm equation. The present study selected the Lou type warfarin pharmacokinetic dosing algorithm equation following comparisons between the equations from multiple studies (1,5,7,18). The warfarin stable dosage equation was as follows: Daily dose of warfarin (mg) = 1.087 + 2.226 x VKORC1(1639AG)$^{\#}$ + 3.844 x VKORC1(1639GG)$^{\$}$ - 1.284 x CYP2C9(*1/*3)$^{\&}$ - 2.182 x CYP2C9(*3/*3)$^{\alpha}$ + 0.221 x CYP4F2(CT)$^{\beta}$ + 0.336 x CYP4F2(TT)$^{\gamma}$ - 0.018 x age (years) + 0.015 x weight (kg) + 0.013 x height (cm) - 0.777 x Amiodarone$^{\lambda}$ - 0.379 x digoxin$^{\sigma}$. Where the following have been applied: $^{\#}$VKORC1(1639AA) = 0, VKORC1(1639AG) = 1; $^{\$}$VKORC1(1639AA) = 0, VKORC1(1639GG) = 1; $^{\&}$CYP2C9(*1/*1) = 0, CYP2C9(*1/*3) = 1; $^{\alpha}$CYP2C9(*1/*1) = 0, CYP2C9(*3/*3) = 1; $^{\beta}$CYP4F2(CC) = 0, CYP4F2(CT) = 1; $^{\gamma}$CYP4F2(CC) = 0, CYP4F2(TT) = 1; $^{\lambda}$used amiodarone = 1, did not use amiodarone = 0; $^{\sigma}$used digoxin = 1, did not use digoxin = 0.

Information collection. According to Table I, 87 patients prescribed with anti-clotting warfarin were randomly divided into the experimental and control groups. Clinical data and blood samples were then collected. The personal data of each participant, including age, sex, height, weight, medical history, hemorrhage history and medication history were recorded. A total of 5 ml blood was obtained from each patient for the present study.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and sequencing. The polymorphism of CYP2C9, VKORC1, and CYP4F2 were detected by PCR-RFLP and DNA sequencing. Peripheral white blood cell genomic DNA were extracted from blood samples using the PAXgene Blood DNA kit (Qiagen, GmbH, Hilden, Germany) (the Japanese supplier being Cosmo Bio Co., Ltd., Tokyo, Japan), according to the manufacturer's instructions. According to the method described previously (20,21), CYP2C9*3 (1075A/C, rs1057910), VKORC1 (1639G/A, rs9923231) and CYP4F2 V433M (rs2108622) were amplified by PCR. Then, the PCR products were digested for 1.5 h at 37˚C with a restriction endonuclease, and analyzed by 2% agarose gel electrophoresis. The DNA sequences of all samples were visualized by ethidium bromide under ultraviolet light using the Molecular Imager Chemi Doc XRS+ System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and analyzed with Quantity One 1D Analysis software (version 4.6.6; Bio-Rad Laboratories, Inc.). Then, all samples were analyzed by DNA sequencing in Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China).

Study strategy. To evaluate the equation in a single-blind manner, the patients were divided into 2 groups without any knowledge of which group they were assigned to. The polymorphism of the 3 genes was determined and the predicted dose of warfarin was calculated according to the Lou type equation, as aforementioned. For the first 3 administrations, all patients in the control group were treated according to the dosages prescribed by an experienced doctor, while all participants in the experimental group were treated using the dosages calculated by the Lou type equation. Then, the dose of warfarin administrated to all patients was gradually adjusted to a stable dose according to the changes in the international standardized ratio (INR). The stable dose means that after the same patient received the same dose, the two consecutive INR values were between 2.0-3.0, and the interval time was >7 days. Following the 50 day experimental period following the first administration, the stable dose, the time to achieve a stable dose and adverse reactions were recorded. In addition, the physician in charge was informed of the study's results and appropriate measures were applied if adverse reactions, including INR >3.5, bleeding, or new thrombosis occurred.

Statistical analysis. All analyses were performed using Stata 12.0 software (StataCorp LP, College Station, TX, USA). Quantitative data are presented as the mean ± standard error of the mean. Measurement data were analyzed using a paired t-test and the enumeration data were analyzed with an χ^2 test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Sequencing analysis. PCR-RFLP was used to detect the genotypes of CYP2C9*3 (1075A/C, rs1057910), VKORC1 (1639G/A, rs9923231) and CYP4F2 V433M (rs2108622). The amplification products of CYP2C9, VKORC1 and CYP4F2 (V433M) were 200, 423 and 491 bp, respectively. Furthermore,

Table I. Comparison of clinical parameters and genotypes between the experimental and control groups.

Clinical parameters	Experimental group (n=42)	Control group (n=45)	P-value
Age (years)	62.5±12.8	61.4±13.2	0.628
Sex (female/male)	17/25	17/28	0.797
Height (cm)	160.2±8.2	159.5±8.6	0.649
Weight (kg)	65.2±10.9	66.8±11.3	0.458
Anticoagulation indications			0.876
Atrial fibrillation (n)	29	32	
Venous thrombosis (n)	5	7	
Pulmonary embolism (n)	3	2	
Valve replacement (n)	5	4	
Combined use of drugs			0.905
Amiodarone (n)	2	2	
Digoxin (n)	8	7	
CYP2C9			0.808
*1/*1 (n)	38	40	
*1/*3 (n)	4	5	
*3/*3 (n)	0	0	
VKORC1			0.563
AA (n)	35	35	
AG (n)	7	9	
GG (n)	0	1	
CYP4F2			0.849
CC (n)	22	22	
CT (n)	16	17	
TT (n)	4	6	

CYP2C9, cytochrome P450 family 2 subfamily C member 9; VKORC1, vitamin K epoxide reductase complex subunit 1; CYP4F2, cytochrome P450 family 4 subfamily F member 2.

the mutated amplification product of CYP2C9*3 (1075A/C) was digested by *Kpn*I into 2 fragments of 180 and 20 bp; the mutated amplification product of VKORC1 (1639G/A) can be digested by *Msp*I into 2 fragments of 207 and 216 bp; and the mutated amplification product of CYP4F2 V433M can be digested by *Pvu*II into 2 fragments of 319 and 178 bp. According to the results of enzyme digestion, there were 3 genotypes of each gene: Mutant homozygote, heterozygote and wild-type (Fig. 1; Table I). All samples were analyzed by DNA sequencing and the results were consistent with those of PCR-RFLP.

Comparison of the clinical parameters. The 87 patients were divided into 2 groups, with 42 participants in the experimental group and 45 participants in the control group. No significant differences were identified in the distribution of the CYP2C9*3, VKORC1 (1639G/A) and CYP4F2 V433M genotypes, age, sex, height, weight and history of disease (Table I) between the 2 groups.

Comparisons of the number of patients exhibited a stable dose, the time for patients to obtain a stable dose and the adverse reactions. According to the obtained statistics, there was a significant difference in the number of patients reaching a stable dose between the 2 groups, with 35 patients (83.3%) in the experimental group and 29 patients (64.4%) in the control group (P=0.046). In addition, it took 18.2±1.7 days for the participants in the experimental group to achieve a stable dose, which was less than that of the control group (27.3±2.0 days). The median time to achieve a stable dose was 11.7±1.1 days for the experimental group and 20.5±1.8 days for the control group, which indicated a statistically significant difference between the two groups (P<0.001). Furthermore, the incidence of adverse reactions in the experimental group was 9.5% (4 cases), which was significantly lower than that of the control group (26.7%; 12 cases; P=0.039). In addition, the average time of developing adverse reactions in the experimental group (43.9±1.6 days) was significantly longer than that of the control groups (38.6±1.5 days; P=0.046).

Comparisons between the predictive dose and actual dose. At the end of the study, 64 participants received the stable dose aggregately. The average dose of 3.4±1.1 mg/day, which was predicted using the Lou type equation, was lower than the actual average dose of 3.5±1.4 mg/day; however, no significant difference was observed between the predicted dose and the actual dose (P=0.313).

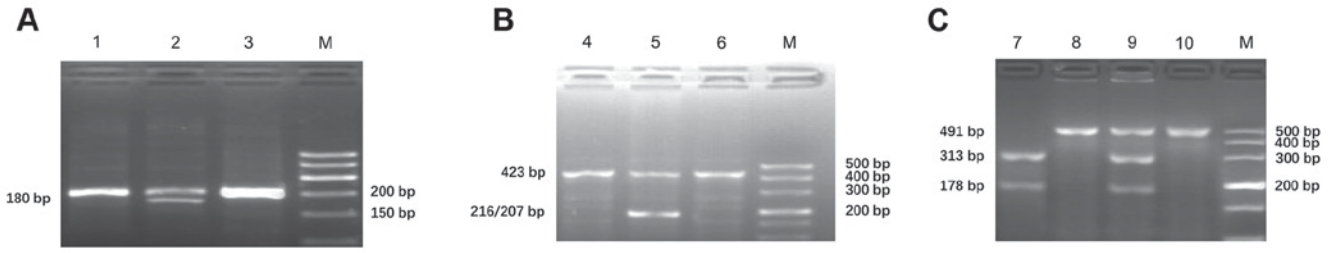

Figure 1. Genotype analysis of (A) CYP2C9*3 (1075A/C, rs1057910), (B) VKORC1 (1639G/A, rs9923231) and (C) CYP4F2 V433M (rs2108622). Lane M, DNA marker; lane 1, wild-type of CYP2C9 (*1/*1); lane 2, heterozygote of CYP2C9 (*1/*3); lane 3, amplification product of CYP2C9 by PCR; lane 4, wild-type of VKORC1(1639A/A); lane 5, heterozygote of VKORC1(1639A/G); lane 6, amplification product of VKORC1 by PCR; lane 7, mutant homozygote of CYP4F2 V433M (TT); lane 8, wild-type of CYP4F2 V433M (CC); lane 9, heterozygote of CYP4F2 V433M (CT); lane 10, amplification product of CYP4F2 V433M by PCR; PCR, polymerase chain reaction; CYP2C9, cytochrome P450 family 2 subfamily C member 9; VKORC1, vitamin K epoxide reductase complex subunit 1; CYP4F2, cytochrome P450 family 4 subfamily F member 2.

Discussion

Individualized treatment refers to the use of individual drugs and also the use of individualized drug dosages based on pharmacogenomics, which has become a popular method for treating cardiovascular illnesses (22). Warfarin is one of the preferred anti-coagulant drugs for the anti-coagulant therapy in patients with atrial fibrillation, deep vein thrombosis, pulmonary embolism, cerebral infarction, radiofrequency ablation, and multiple types of cardiomyopathy at present (23). Previous studies have demonstrated that non-genetic factors including sex, body weight, body surface area, age, number of increasing INR drugs, smoking habit, preoperative stroke history and hypertension were minor determinants of warfarin stable dosage (6,19,24). Thus, the problem of how to determine the stable dosage and how to maintain the dose accurately and conveniently should be addressed.

Some previous studies focusing on pharmacogenomics have indicated that the single nucleotide polymorphism (SNP) of CYP2C9*3 and VKORC1(1639G/A) may affect the pharmacodynamics and pharmacokinetics of warfarin (3,4,8-11,14,22, 24,25). In addition, other previous studies have suggested that the SNP of CYP4F2 V433M may be a determinant of warfarin's pharmacodynamic and pharmacokinetic properties (21).

Although numerous equations for predicting a stable dose of warfarin have been certificated, their practical application value has differed (4,5,7,19,22,26). A number of verification tests performed for these equations did not produce perfect results (27-29). However, the results were worse still in clinics, as many studies from China were only based on a single disease, including atrial fibrillation, pulmonary embolism or valve replacement; particularly in the cardiovascular departments of primary hospitals, where warfarin was often used for atrial fibrillation, and in larger hospitals, where warfarin was used for valve replacements (30,31). In some cases, combining warfarin with digoxin treatment has produced better results in patients with rheumatic heart disease or chronic heart failure; however, patients who also had valve replacement may require the combination of warfarin with amiodarone (32-35). Therefore, the warfarin stable dose prediction equation derived by taking into account a variety of factors that affect the stability of warfarin dose may be the best option for patients and doctors. Following comparison with other equations, it was demonstrated that the Lou type equation used in the present study may have greater practical value in the Chinese Han population (1,5,7,21,26-28).

In the present study, the value of the Lou type warfarin pharmacokinetic dosing algorithm equation was verified through a randomized controlled prospective study of Han Chinese patients in Zhejiang. The results indicated that the experimental group yielded a greater number of cases reaching a stable dose and took less time to achieve a stable dosage than the control group. Therefore, using the Lou type equation may significantly shorten the dosage adjusting time, facilitate an effective and stable drug concentration, reduce detection by INR and even decrease therapeutic costs.

Furthermore, the incidence of adverse reactions in the control group was 26.7% (12 cases), which was significantly higher than that of the 9.5% in the experimental group (4 cases). In addition, the experimental group took 43.9±1.6 days to develop adverse reactions, while these reactions were observed in the control group following 38.6±1.5 days. Thus, using the Lou type equation may reduce the incidence of side effects and delay the occurrence of adverse reactions, resulting in a safer clinical application of warfarin.

In the present study, 65 participants received the stable dose aggregately. The average dose of 3.5±1.1 mg/day, which was predicted by the Lou type equation, was lower than the actual average dose of 3.5±1.4 mg/day, however, no significant difference was found between the two groups. The results of the present study are indicative of the strong application value of the Lou type warfarin pharmacokinetic dosing algorithm equation for treating Han Chinese patients.

Although the present study yielded favorable results, it did have some limitations. For individuals, the predicted stable dose was an estimate that may have a large discrepancy with the actual value, likely leading to an unnecessary clinical risk. Therefore, the predicted stable dose should be considered as a reference in the process of adjusting dosages. Doctors or physicians should be more concerned with the monitored INR. In terms of estimating eating habits, drug use and health should not be underestimated. As the present study did not adjust dosages for factors including eating habits, socioeconomic status and patients' cognition regarding warfarin, a local large-scale study is required to yield more accurate data.

In conclusion, the application of Lou type warfarin pharmacokinetic dosing algorithm equation markedly shortened the adjustment time and reduced the occurrence of adverse

reactions, which suggested that it may have great value in clinical drug application.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

JGJ designed the study, performed the experiments, analyzed the data and wrote the manuscript. NNJ, JLL, XPG and XMD collected the cases. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Jinhua Hospital of TCM Affiliated to Zhejiang University of Traditional Chinese Medicine (Jinhua, Zhejiang, China). Written informed consent was obtained from all participants.

Consent for publication

Written informed consent was obtained from all participants.

Competing interests

All authors declare that they have no competing interests.

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