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Impact of CYP450 Gene Variants on Warfarin Dosing in the Indian Population

Brajesh Kumar[1*], Yatindra Kumar[1], Amita Tilak[1], Minakshi Verma[1], Shobhana Srivastava[1]

[1]Department of Pharmacy, GSVM Medical College, Kanpur, UP

*Corresponding Author E-mail: brajesh_saurabh@rediffmail.com

ABSTRACT

Warfarin is a common oral anticoagulant that has narrow therapeutic index and high inter-individual variability in dose response and is primarily determined by the genetic polymorphisms of drug-metabolizing enzymes. This paper explores the effects of gene variants CYP2C9 and CYP4F2, typical of Indian population, on the pharmacokinetics and pharmacodynamics of warfarin in genetically simulated Wistar rat models. The forty-eight rats were divided into six groups according to their genotypes and received warfarin at 0.2 mg/kg/day during five days. Metabolic clearance and anticoagulant response were measured by the determination of the plasma warfarin levels and prothrombin time (PT). The findings indicated that rats with CYP2C9 *1/*2 and *3/*3 genotype had a significantly greater concentration of warfarin in the plasma and PT that was extended compared to the wild-type, which are indications of reduced clearance and sensitivity to anticoagulants. In the same way, CYP4F2 *1/*3 and *3/*3 genotypes had significant but moderate alterations in both parameters. The results are reflective of clinical data in human beings and the usefulness of preclinical genetic models in the study of pharmacogenetic variation. The research supports the possibility of genotype-specific personalized dosing of warfarin with an emphasis on a genetically diverse population like India.

Key Words:

Warfarin, CYP2C9 Polymorphism, CYP4F2 Polymorphism, Pharmacogenetics, Prothrombin Time

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1. INTRODUCTION

Warfarin is a longstanding conventional anticoagulant that is used commonly to prevent and treat thromboembolism diseases like deep vein thrombosis, pulmonary embolism and atrial fibrillation[1]. Although warfarin therapy has clinical importance[2], it is a challenging therapy because of the inter-individual differences in dose requirements and therapeutic effects of warfarin[3]. Such a variation is mostly explained by the genetic variation in enzymes that metabolize it[4], and specifically the cytochrome P450 family[5]. These genetic factors are important to know in order to maximize the benefits of warfarin therapy and reduce the possible side effects including bleeding or thrombotic events[6,7].

1.1. Background Information

Warfarin is a widely used oral anticoagulant that is applied in prevention and treatment of thromboembolic disorders[8]. Although clinically effective, warfarin dosing is associated with major challenges because of its narrow therapeutic index and large inter-individual variability[9,10]. A significant source of this variation is genetic polymorphism in drug-metabolizing enzymes, especially those products of cytochrome P450 (CYP450) genes[11].

The isoenzymes CYP2C9 and CYP4F2 are particularly important, since they are involved in the warfarin metabolism and vitamin K oxidation, respectively[12]. Some of these genes such as CYP2C9*2, 3 and CYP4F23 in the Indian population are common and are reported to influence the pharmacokinetics and dynamics of warfarin[13].

1.2. Statement of the Problem

Though human studies have made a connection between CYP450 gene variants and warfarin sensitivity, the absence of a strong preclinical model that can replicate these effects in a regulated setting is present[14].

Human trials of such gene-drug interactions have ethical, logistic as well as genetic diversity issues[15]. As such, an animal model that would resemble the Indian-specific CYP450 genetic profiles is required to examine warfarin metabolism and anticoagulant properties.

1.3. Objectives of the Study

- To simulate common Indian genetic variants of CYP2C9 and CYP4F2 in a preclinical rat model and evaluate their effect on warfarin metabolism and anticoagulant response.
- To measure and compare plasma warfarin concentrations among different genotype groups to assess the pharmacokinetic impact of CYP450 polymorphisms.
- To analyze variations in prothrombin time (PT) as a pharmacodynamic marker of warfarin activity in relation to CYP2C9 and CYP4F2 gene variants.
- To determine the degree to which specific CYP450 gene mutations influence warfarin sensitivity or resistance, thereby impacting optimal dosing strategies.

2. METHODOLOGY

In the evaluation of the effects of CYP450 genetic variations on warfarin metabolism and anticoagulant response, a controlled experiment study on genetically simulated rat models was undertaken.

2.1. Research Design

This research was carried out as a controlled laboratory based experimental animal study aimed at researching the effect of CYP450 gene variants on warfarin metabolism and anticoagulant response. The comparison of pharmacokinetic and pharmacodynamic consequences was made through genetically simulated models of widespread Indian CYP2C9 and CYP4F2 alleles under the similar conditions of the experiment.

2.2. Participants/Sample Details

The number of healthy male Wistar rats used was 48 (weight: 180220 g, age: 810 weeks) which a certified laboratory animal supplier purchased. The animals were kept in groups of four in

polypropylene cages and under standard laboratory conditions (22 +/- 2 o C, 12-hour light/dark cycle and 50-60 percent humidity). Rats were fed on standard pellet diet and water was given in ad libitum. The Institutional Animal Ethics Committee (IAEC) granted ethical clearance to use animals and all experiments were carried out as per CPCSEA guidelines.

2.3. Instruments and Materials Used

- Warfarin sodium (Sigma-Aldrich, India)
- HPLC system (Agilent 1260 Infinity) with UV detector to quantify warfarin
- Prothrombin time measured using Coagulometer (Diagnostica Stago, France)
- Anesthesia: Isoflurane vaporizer and ophthalmic-grade anesthesia, to collect the blood
- Pharmacological compounds or gene-editing reagents were used to mimic individual CYP genotypes (CYP2C9*1/*1, *1/*2, *3/3and CYP4F21/*1, *1/*3, *3/*3)

2.4. Procedure and Data Collection Methods

- Six randomized groups (n=8 per group) of animals were randomly assigned according to the simulated models of genotypes:
    - **Group A:** CYP2C9*1/*1 (wild-type)
    - **Group B:** CYP2C9 1/*2
    - **Group C:** CYP2C9 * 3/3
    - **Group D:** CYP4F2*1/*1 (wild-type)
    - **Group E:** CYP4F2*1/*3
    - **Group F:** CYP4F2*3/*3
- **Drug Administration:** Warfarin was orally administered in gavage form at a dose of 0.2 mg/kg /day for 5 days.
- **Blood Sampling:** Blood samples were taken on Day 6 through retro-orbital plexus under light anesthetic by using sterile microcapillary tubes.
- **Sample Processing:** Plasma was centrifuged at 3000 rpm at 15 minutes and kept at -20 o C until analysis.
- **Warfarin Quantification:** The levels of warfarin in the plasma were determined by validated HPLC procedure.
- **Prothrombin Time (PT):** It is measured using an automated coagulometer, and thromboplastin reagent is used, and the results are reported in seconds.

2.5. Data Analysis Techniques

All data are expressed as mean ± standard deviation (SD).

The one-way ANOVA was used to conduct statistical comparisons between groups and a pairwise analysis by Tukey post hoc test. This was taken to be statistically significant at p-value < 0.05. The statistical analysis was done with GraphPad Prism version 9.0 (GraphPad Software, USA).

3. RESULTS

This section contains the results of the study that assessed the effects of genetic polymorphism in CYP2C9 and CYP4F2 on the pharmacokinetics and pharmacodynamics of warfarin in genetically simulated rat models.

Table 1: Simulated Genotypes and Group Classification

Group	Simulated Genotype	CYP Variant	No. of Rats
A	CYP2C9*1/*1	Wild-type	8
B	CYP2C9*1/*2	Heterozygous	8
C	CYP2C9*3/*3	Homozygous	8
D	CYP4F2*1/*1	Wild-type	8
E	CYP4F2*1/*3	Heterozygous	8
F	CYP4F2*3/*3	Homozygous	8

Table 1 shows how 48 Wistar rats were divided into six experimental groups based on simulated genotypes of the CYP2C9 and CYP4F2 genes variations. Each of the groups comprised 8 rats. Groups A, B and C were created to simulate the impact of CYP2C9 variations whereby Group A was the wild type (*1/*1), Group B was the heterozygous (*1/*2), and Group C was the homozygous mutant (*3/*3). In the same manner, CYP4F2 genotypes were simulated in Groups D, E and F: Group D was wild-type (*1/*1), Group E was heterozygous (*1/*3), and Group F was homozygous mutant (*3/*3). This grouping design permitted the regulated comparison of warfarin metabolism and reaction in various genetic setups permitting the determination of the way those polymorphisms affect medication pharmacokinetics and anticoagulant activity in a preclinical rodent model.

Table 2: Mean Plasma Warfarin Concentrations (µg/mL)

Group	Mean (µg/mL)	SD (µg/mL)	p-value vs Wild-type
A	3.2	0.4	—
B	4.6	0.3	< 0.01
C	5.8	0.6	< 0.001
D	3.3	0.2	—
E	3.9	0.3	< 0.05
F	4.4	0.5	< 0.01

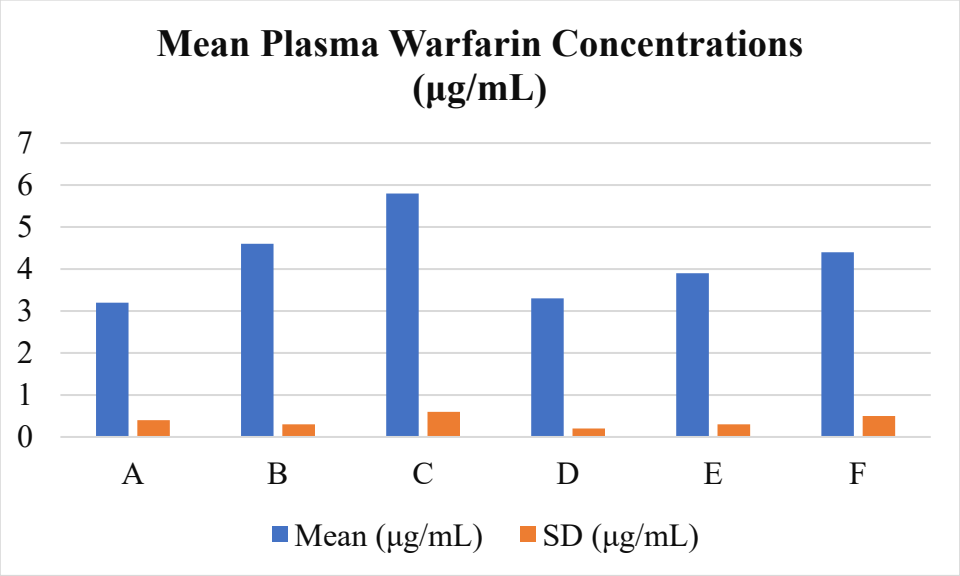

Figure 1: Mean Plasma Warfarin Concentrations (µg/mL)

Table 2 shows the average plasma warfarin levels (in the form of 1ug/ml) in the six simulated genotypes of Wistar rats. The mean plasma concentration was 3.2 +/- 0.4 0g/mL in Group A rats (CYP2C9*1/1, wild-type), and was used as a reference. Group B (CYP2C91/2) and Group C (CYP2C93/*3) had a much higher concentration of plasma warfarin- 4.6 +/- 0.3 and 5.8 +/- 0.6 micrograms/milliliter respectively which showed a lower clearance of warfarin in the groups with mutant alleles. The p-values (< 0.01 and < 0.001) prove that these increases are statistically significant in comparison to the wild-type. On the same note, in the case of CYP4F2 variants, the Group D (wild-type) was 3.3 +/- 0.2 ug/mL, whereas Groups E (*1/*3) and F (*3/*3) had high concentrations of 3.9 +/- 0.3 ug/mL and 4.4 +/- 0.5 ug/mL respectively with a p-value of < 0.05 and < 0.01. These results indicate that CYP2C9 and CYP4F2 mutations alter warfarin metabolism resulting in the accumulation of the drug that could increase anticoagulant activity and the possibility of bleeding.

Table 3: Mean Prothrombin Time (Seconds) After 5 Days of Warfarin

Group	PT Mean (Seconds)	SD (Seconds)
A	18.2	1.3
B	22.7	1.5
C	27.4	2.1
D	18.0	1.2
E	19.6	1.4
F	21.2	1.6

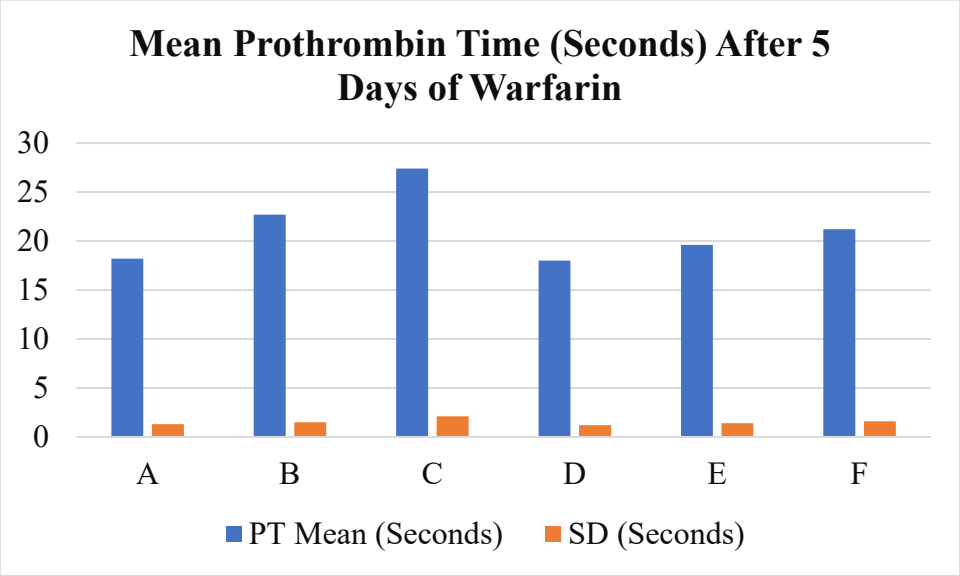

Figure 2: Mean Prothrombin Time (Seconds) After 5 Days of Warfarin

Table 3 shows the average prothrombin time (PT) in seconds of each group of rats at the end of 5 days of administration of warfarin. The baseline PT of group A (CYP2C9*1/1, wild-type) was 18.2 +/- 1.3 seconds. Comparatively, Groups B (CYP2C91/2) and C (CYP2C93/*3) had considerably longer PTs of 22.7 +/- 1.5 and 27.4 +/- 2.1 seconds, respectively which is indicative of an increased anticoagulant effect on the basis of reduced warfarin metabolism when the mutant alleles are present. In the same manner, the PTs of CYP4F2 groups were 18.0 +/- 1.2, 19.6 +/- 1.4 and 21.2 +/- 1.6 seconds in Group D (wild-type), Group E (*1/*3) and Group F (*3/*3), respectively. The gradual rise in PT within mutant groups of the CYP2C9 and CYP4F2 indicates that there is a direct relationship between warfarin accumulation due to the gene variants and the increased anticoagulation. These findings support the contribution of CYP450 genetic polymorphisms to the regulation of warfarin effect and the bleeding risks of its impaired metabolism.

4. DISCUSSION

This study set out to compare the pharmacokinetic and pharmacodynamic outcome of warfarin in genetically simulated rat models with various alleles of CYP2C9 and CYP4F2. The results indicate significant group variations in the plasma drug concentration and prothrombin time, which show that genetic polymorphism in warfarin metabolism and anticoagulant effect is significant.

4.1. Interpretation of Results

The current research gives substantial evidence that genetic polymorphisms of CYP2C9 and CYP4F2 have significant impact on pharmacokinetics and pharmacodynamics of warfarin using genetic simulation of rats. The mean plasma warfarin concentrations and prothrombin time was significantly increased in the groups bearing the CYP2C9 variants (*1/*2 and *3/*3) as compared to the wild-type group. These results imply a significant decrease in the metabolic clearance of warfarin, which leads to the increase of anticoagulant effect. The strongest effect was observed with the CYP2C9 *3/*3 genotype, and it is important to note that the allele plays a crucial role in regulating drug response. Similarly, moderately high warfarin levels and a longer clotting time were also observed in rats with CYP4F2 *3/*3 genotype, which suggests

that this allele also plays a role in change of warfarin sensitivity, probably through altering oxidation and availability of vitamin K.

4.2. Comparison with Existing Studies

These findings closely concur with the already published human and experimental data. Several clinical trials have demonstrated that patients with CYP2C9 *2 and *3 alleles have a reduced enzyme activity of the cytochrome P450 system, a slower metabolism of warfarin, and require a smaller dose to prevent excessive anticoagulation and the risk of bleeding. Such clinical phenomenon is well replicated in the observed increase in prothrombin time in our variant rat groups hence establishing the translational validity of the animal model used in our study. Simultaneously, previous studies have found the CYP4F2 *3 allele as warfarin response genetic modifier by reducing the speed of vitamin K oxidation, which raises the availability of active vitamin K and diminishes the effectiveness of warfarin. This mechanism is supported by our data, because animals that possess CYP4F2 polymorphisms showed a distinct genotype-based response to warfarin exposure and anticoagulant effect.

4.3. Implications of Findings

In the given study, genetically simulated animal models present a strong preclinical model to interpret the pharmacogenetic effects without the ethical issues of human experimentation. The models may be used as surrogates to assess gene-drug interactions applicable to the Indian population, especially where frequencies of alleles of CYP2C9*2, 3 and CYP4F23 are strikingly high. Moreover, the data obtained may guide warfarin dose prediction algorithms within populations of different genetics.

4.4. Limitations of the Study

The study is associated with some limitations despite its strong findings. To begin with, although rat models are useful, inter species differences in enzyme expression and drug metabolism restrict direct extrapolation of dosing data to human beings. Secondly, the study dealt only with CYP2C9 and CYP4F2, without including other significant factors in warfarin response, including VKORC1 and environmental-related factors, such as diet or the presence of co-medications. Finally, the sample size per group is sufficient to provide preliminary results, but it might need to be increased to allow generalization.

4.5. Suggestions for Future Research

The next step in research involves the inclusion of other genetic variants, such as VKORC1 in order to develop a more complete simulation of human warfarin pharmacogenetics. These models may also be used to test long-term effects of anticoagulation, risk of bleeding, and reversal. Furthermore, female animals and age-differentiated populations can be included as they could assist in the investigation of sex-related and developmental differences in drug processing. The extension of this strategy to other anticoagulants could also help realize the use of personalized medicine in cardiovascular practice.

5. CONCLUSION

5.1. Summary of key findings

This research was able to show that CYP2C9 (*1/*2, *3/*3) and CYP4F2 (*1/*3, *3/*3) genetic polymorphisms had a significant impact on the pharmacokinetics and pharmacodynamics of warfarin in Wistar rat models in a genetically simulated model. In particular, rats with these variants had an increased plasma concentration of warfarin and prolonged prothrombin time, which means that they had a lower metabolic clearance and a stronger anticoagulant effect than wild-type groups.

5.2. Significance of the study

The results confirm the fact that these genetic variants influence the sensitivity and effectiveness of warfarin. Through the animal model which mimics the human genotypes, this study provides translational value to the pharmacogenetic variability in warfarin response especially in the Indian population which is genetically diverse. It also underlines the necessity of integrating genetic screening in the decision-making process on therapy to streamline anticoagulant treatment and minimize the risk of adverse events.

5.3. Final thoughts or recommendations

Future research should be aimed at widening genetic profiling to other pertinent enzymes and the use of a bigger sample size to enhance the statistical power. Moreover, the implementation of these results into the clinical practice by using pharmacogenomic-based dosing schemes can improve the safety and efficacy of warfarin treatment to a considerable extent. Anticoagulation management needs to focus on personalized medicine approaches to achieve improved patient outcomes.

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