International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Special Issue 5, 2022

Original Article

PREDICTION OF THE EFFECT OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN THE CYP2C9 ON WARFARIN METABOLISM BY *IN SILICO* STUDY

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Received: 14 Sep 2022, Revised and Accepted: 10 Nov 2022

ABSTRACT

Objective: This study aimed to predict the effect of SNPs CYP2C9 s on the metabolic activity of S-warfarin in the body.

Methods: Molecular modeling was performed to obtain SNPs CYP2C9 and molecular docking was performed to predict the effect of SNPs CYP2C9 on the metabolic activity of S-warfarin.

Results: The results showed that wild-type CYP2C9 had the strongest binding affinity (ΔG: -9.76 kcal/mol), indicating that wild-type CYP2C9 had the best metabolic activity compared to SNPs CYP2C9. There was a decrease in hydrogen bond formation in SNPs CYP2C9 and an increase in the distance between C7 S-warfarin and Fe-Heme in CYP2C9 SNPs when compared to wild-type CYP2C9

Conclusion: The decrease in binding affinity, decrease in hydrogen bond formation, and an increase in the distance between C7 S-warfarin and Fe-Heme on SNPs CYP2C9 indicated that SNPs CYP2C9 had decreased metabolic activity against S-warfarin which led to an increased risk of bleeding.

Keywords: Warfarin, CYP2C9, SNPs CYP2C9, *In silico*, Molecular modeling, Molecular docking

DOI: https://dx.doi.org/10.22159/ijap.2022.v14s5.14 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

In 2018, Baseline Health Research stated that the prevalence of cardiovascular disease in Indonesia was increasing every year. There have been many therapies for cardiovascular disease, one of which is pharmacological therapy using anticoagulant drugs. Warfarin is a coumarin-derived oral anticoagulant used for embolization prophylaxis of atrial fibrillation and rheumatic heart disease, prophylaxis and treatment of pulmonary embolism and venous thrombosis, prophylaxis after prosthetic heart valve insertion, and transient cerebral ischemic attacks [1].

As an oral anticoagulant, warfarin is widely used despite the wide variability in dosage between individuals. Genetic factors are the main reason individuals have varying maintenance doses of warfarin. The genetic factor that plays a role in warfarin dose variability is the Cytochrome P450 Family 2 Subfamily C Member 9 (CYP2C9) gene [2]. In warfarin, CYP2C9 works by catalyzing the oxidation reaction of the active form of warfarin, namely S-warfarin, to inactive metabolites, namely 6-hydroxy and 7-hydroxy warfarin. CYP2C9 is reported to have many Single-Nucleotide Polymorphisms (SNPs). CYP2C9 or CYP2C9 wild type (WT) is a gene that does not undergo mutations and still function as it should. Meanwhile, SNPs CYP2C9 are CYP2C9 which undergo changes in the single nucleotide base arrangement, which causes genetic variation in a population. Examples of the forms of SNPs CYP2C9 that occur are CYP2C9*2 (144 Arg>Cys), CYP2C9*3 (359 Ile>Leu), CYP2C9*5 (360 Asp>Glu), CYP2C9*11 (Arg>Trp 335), CYP2C9* 12 (489 Pro>Ser), and CYP2C9*13 (90 Leu>Pro) [3].

According to an article conducted by Pavani *et al.* in 2015, concluded that CYP2C9*2 and CYP2C9*3 greatly affect warfarin dose variability because they can reduce CYP2C9 enzyme activity, causing a decrease in the metabolism of warfarin in the body [2]. Most warfarin dosing algorithms only include the effects of CYP2C9*2 and CYP2C9*3, but previous studies have found that CYP2C9*5, CYP2C9*11, CYP2C9*12, and CYP2C9*13 are also likely to cause decreased CYP2C9 enzyme activity resulting in decreased metabolism by CYP2C9.

Based on the above, a study was conducted to determine how the effect of CYP2C9 SNPs on warfarin metabolism in the body by *in silico* technique, comprised of molecular modeling of CYP2C9 SNPs and molecular docking. The interpretation of the results was carried out by comparing three parameters, namely the binding affinity, the interaction of hydrogen bonds formed between the protein and the ligand, and the distance between C7 S-warfarin and Fe-Heme present in the protein. These three parameters are known to play a role in the metabolic ability of CYP2C9.

MATERIALS AND METHODS

Materials

The hardware used is a personal laptop with specifications AMD Ryzen 5 4500U 2.3 GHz up to 4.0 GHz, 8 Gigabyte RAM soldered DDR4-3200, 512 Gigabyte SSD storage. The software used is BIOVIA Discovery Studio 2017, ChemDraw Ultra and Chem3D, AutoDock 4.2.6 (The Scripps Research Institute), PyMOL 2.5, and LigandScout 4.4.3.

Molecular modeling of SNPs CYP2C9

Molecular modeling of the 3-dimensional structure of SNPs CYP2C9 was performed using PyMOL 2.5 with CYP2C9 WT (PDB ID: 1OG5) as the template. Molecular modeling was carried out by substituting amino acid residues in CYP2C9 WT into amino acid residues in CYP2C9 SNPs. For example, in CYP2C9*3, isoleucine changes to leucine at position 359, then isoleucine is substituted in CYP2C9 WT to become leucine at position 359. Furthermore, in CYP2C9*5, an aspartate changes to glutamine at position 360; in CYP2C9*11 changes from arginine to tryptophan at position 335; in CYP2C9*12 changes occur from proline residues to serine at position 489, and CYP2C9*13 changes from leucine to proline at position 90 This molecular modeling was carried out using the "wizard" and "mutagenesis" commands in the PyMOL 2.5 program. The 3-dimensional structure of a protein that has substituted one of its amino acids is then stored in. pdb format [4].

Molecular docking

The preparation of the three-dimensional structure of the ligand, S-warfarin, was carried out by minimizing the energy of S-warfarin using Chem3D. The three-dimensional structure of S-warfarin that was created was then optimized using AutoDock 4.2.6. The preparation of the three-dimensional structure of the target protein, namely CYP2C9 WT, CYP2C9*3, CYP2C9*5, CYP2C9*11, CYP2C9*12, and CYP2C9*13 was carried out by removing water molecules and natural ligands using BIOVIA Discovery Studio 2017 and then optimized using AutoDock 4.2.6.

Validation method was carried out by re-docking between CYP2C9 WT and its natural ligand using the AutoDock 4.2.6 program. Molecular docking was performed with a grid box size of 60 × 80 × 60 Å with a distance of 0.375 Å and the grid box coordinates were centered on the natural ligand. Then the parameter analyzed is the RMSD (Root Mean Square Deviation) value with the condition ≤ 2Å [5].

Molecular docking simulations between warfarin as a ligand and CYP2C9*3, CYP2C9*5, CYP2C9*11, CYP2C9*12, and CYP2C9*13 as target proteins were carried out one by one using AutoDock 4.2.6. Molecular docking was carried out using a grid box measuring 60 × 80 × 60 Å with the distance of 0.375 Å [5].

Interpretation was carried out by comparing three parameters, namely binding affinity, distance between C7 S-warfarin and Fe-Heme, and hydrogen bond interactions between CYP2C9 WT and SNPs CYP2C9. The interpretation of the results was carried out using BIOVIA Discovery Studio 2017 and LigandScout 4.4.3 [5].

RESULTS AND DISCUSSION

Molecular modeling of SNPs CYP2C9

Molecular modeling of SNPs CYP2C9 performed using the PyMOL 2.5 program aims to produce a 3D structure of CYP2C9 SNPs which is then used for molecular docking. Molecular modeling was performed by substitution of amino acid residues on CYP2C9 WT to produce five forms of SNPs CYP2C9, namely CYP2C9*3, CYP2C9*5, CYP2C9*11, CYP2C9*12, and CYP2C9*13.

Molecular docking simulation

Validation method of molecular docking is a step carried out before the molecular docking between the target protein and the ligand. The validation results were observed based on the RMSD (Root Mean Square Deviation) value. From the research, it was found that the RMSD of CYP2C9 WT used was 0.64 Å, where a validation method was said to be eligible if the RMSD value obtained was≤ 2Å [5]. Thus, the results of the validation method between CYP2C9 WT and natural ligands have met the requirements and can be continued to the next stage. In the validation method also obtained a coordinate called the grid box coordinates, namely x (-20.236), y (86.761), and z (38.657).

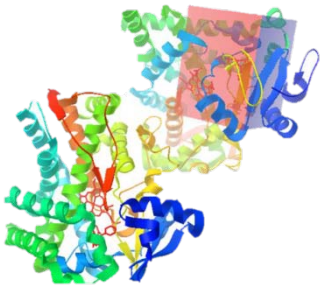

Fig. 1: CYP2C9 WT active pocket indicated by red and blue boxes. The box is located at x (20.236), y (86.761), and z (38.657) coordinates, measuring 60 × 80 × 60 Å with a distance of 0.375Å. The box is centered on the Phe476, which is the key residue

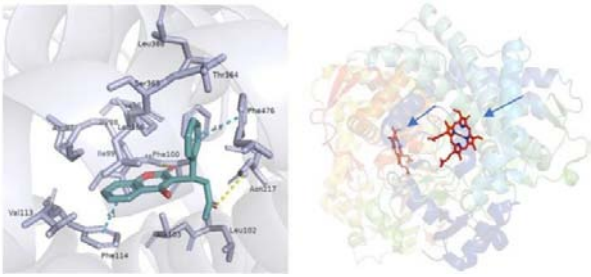

Fig. 2: The interaction between CYP2C9 WT and S-warfarin (Left) and the location of Heme on CYP2C9 WT indicated by blue arrows (Right)

From the results of the two-dimensional visualization of CYP2C9 WT with S-warfarin, it was found that S-warfarin resides in the hydrophobic pocket of CYP2C9 WT with several amino acid residues involved. Specifically, two pi-pi stacking interactions were formed between Phe476 and Phe114 residues with the phenyl group of S-warfarin indicated by the blue dotted line. The Phe476 residue is a key residue because it has conformational mobility, which is very important for the movement of S-warfarin to heme at CYP2C9 [2]. Hydrogen bonding interactions occur between the carbonyl group of S-warfarin with residues of Phe100 and Asn217, which are indicated by the yellow dotted line. This is in accordance with William *et al.* (2003) who described S-warfarin in the hydrophobic pocket of CYP2C9, the formation of pi-pi stacking interactions with Phe476 and hydrogen bonds with Phe100 [6]. In CYP2C9, there is Heme which functions as a cofactor by being involved in the catalysis of oxidation-reduction reactions [7].

Table 1: Molecular docking results between CYP2C9 WT, CYP2C9*3, CYP2C9*5, CYP2C9*11, CYP2C9*12, and CYP2C9*13 with S-warfarin

Target protein	ΔG (kkal/mo l)	Ki (nM)	Amino acid residues	
			Hydrogen bond	Others interaction
CYP2C9 WT	-9.76	66.74	Phe100, Asn217	Phe476, Phe114, Phe100, Ala103, Leu366, Pro367
CYP2C9*3	-8.89	306.33	Asn217	Phe114, Phe100, Phe476, Arg97, Ile99, Ala103, Leu366, Pro367
CYP2C9*5	-8.41	688.48	Phe100	Phe114, Phe100, Phe476, Arg97, Ala103, Leu366, Pro367
CYP2C9*11	-8.87	316.84	Phe100	Phe114, Phe100, Phe476, Arg97, Ala103, Leu366, Pro367
CYP2C9*12	-8.90	301.20	Phe100	Phe476, Phe114, Arg97, Ile99, Ala103, Leu366, Pro367

Gibbs free energy is the main parameter related to protein-ligand binding affinity. From the results of molecular docking, the overall Gibbs free energy is negative. CYP2C9 WT gave the most negative Gibbs free energy value, indicating that CYP2C9 WT gave the best metabolic activity against S-warfarin when compared to SNPs CYP2C9.

Another parameter that can be seen is hydrogen bonding. Hydrogen bonding makes a significant contribution to the protein-ligand binding affinity. In protein-ligand interactions, the more hydrogen bonds formed, the more stable the protein-ligand complex is formed and the stronger the interaction between the ligand-protein. Based on the molecular docking study conducted by Pavani *et al.* in 2015, which carried out the molecular bonding between CYP2C9*2 and CYP2C9*3 with S-warfarin, it was concluded that decreasing hydrogen bond formation can reduce protein-ligand binding affinity [2]. At CYP2C9 WT, two hydrogen bonds were formed between the carbonyl group of

S-warfarin and the amino acid residues Phe100 and Asn217. Meanwhile, the five SNPs CYP2C9 experienced a decrease in hydrogen bond formation, which caused a decrease in protein-ligand binding affinity and caused a decrease in CYP2C9 metabolic activity.

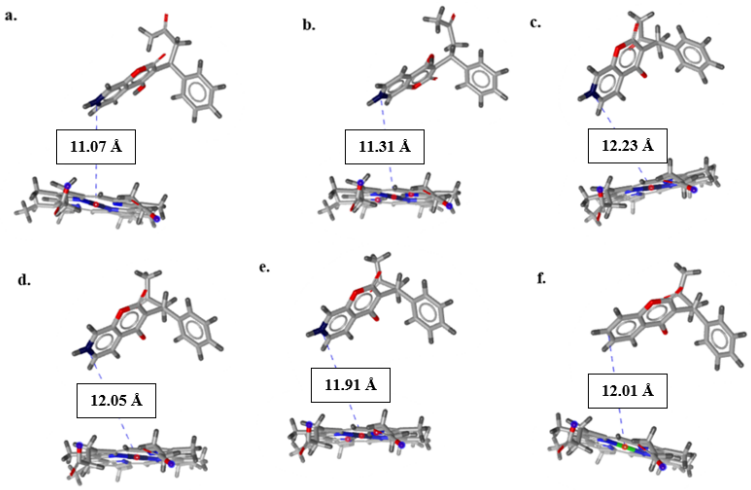

Fig. 3: Distance between C7 S-Warfarin and Fe-Heme located at (a) CYP2C9 WT, (b) CYP2C9*3 (359 Ile>Leu), (c) CYP2C9*5 (360 Asp>Glu), (d) CYP2C9*11 (335 Arg>Trp), (e) CYP2C9*12 (489 Pro>Ser), and (f) CYP2C9*13 (90 Leu>Pro) calculated using the Ligand Scout 4.4.3 program

The next parameter is the distance between C7 S-warfarin and Fe-Heme on CYP2C9. An increase in distance causes undesirable interatomic interactions, which in turn causes a decrease in activity in the SNPs CYP2C9. The potential distance for the hydroxylation process is in the range of 10-11 Å [8]. From the results of molecular docking of CYP2C9 WT with S-warfarin, the distance formed between C7 S-warfarin and Fe-Heme was 11.07Å. Meanwhile, the five SNPs CYP2C9 experienced an increase in distance in the range of 0.24–1.16 Å, which indicates that the SNPs CYP2C9 may have decreased metabolic activity due to a decrease in the hydroxylation process.

In silico study predicted a decrease in binding affinity and an increase in S-warfarin distance to Fe-heme in five SNPs CYP2C9. Stronger bond affinity and shorter distance between C7 S-warfarin and Fe-Heme results in more significant metabolic activity of the CYP2C9 WT enzyme. The residues that play a role are Phe476, Phe100, and Asn217. Phe476 helps position S-warfarin close to Fe-Heme so that S-warfarin will be hydroxylated. Phe100 and Asn217 form hydrogen bonds with S-warfarin, increasing the bond affinity between S-warfarin and CYP2C9 [8].

CONCLUSION

The SNPs CYP2C9 used had decreased binding affinity, decreased hydrogen bond formation, and increased distance between C7 S-warfarin and Fe-Heme, which indicated that CYP2C9 SNPs had decreased metabolic ability compared to CYP2C9 WT. Decreased metabolic activity of S-warfarin by CYP2C9 SNPs may increase the risk of bleeding. This study can be used as scientific information on the effect of CYP2C9 SNPs on the metabolic activity of S-warfarin in the body. However, the results of *in silico* studies need to be verified through molecular dynamics simulations, *in vitro* and *in vivo* studies.

ACKNOWLEDGMENT

The Authors are grateful to the laboratory research assistant at Laboratory of analytical pharmacy Universitas Padjadjaran for technical support, including the material and equipment used.

FUNDING

The authors are grateful to the Rector of Universitas Padjadjaran for funding this study via the DRPM internal grant of RDPD No 2203/UN6.3.1/PT.00/2022.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT INTERESTS

The authors declared no conflict of interest

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