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Original Article

EFFECT OF NON-SYNONYMOUS SINGLE-NUCLEOTIDE POLYMORPHISM OF HUMAN CARBOXYL ESTERASE 1 ON THE BIOACTIVATION OF DABIGATRAN ETEXILATE

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ABSTRACT

Objective: Dabigatran Etexilate is an oral acting direct thrombin inhibitor used for prophylaxis of cardioembolic events in non-valvular atrial fibrillation patients. Genetic polymorphisms in hCES1 gene can significantly alter the conformations of the enzyme drug binding and impair the catalytic ability. Hence this study is performed to determine the effect of single nucleotide variations on dabigatran activation by hCES1.

Methods: Energy minimization was performing using YASARA server. Dihedral angles of the modeled targets were visualized using Ramachandran Plot. We performed molecular docking analysis with Autodock 4.2. Three-dimensional grid was constructed using Autogrid. Docked complexes were visualized using Pymol Viewer.

Results: Significant differences in the binding energy and conformations of Dabigatran in the active site of the target was observed between the Ser75Asn, Ala158Val, Asp203Glu, Ala269Ser and Thr290Met variants. The catalytic triad was completely or partially disrupted in the variants suggestive of altered enzyme activity.

Conclusion: The binding energy of Dabigatran with the mutant (79Ala, 221Ala, 354Ala and 468Ala) was found to be less than that of the wild type. This indicates that the presence of functional non-synonymous polymorphism in the hCES1 significantly alters the binding of Dabigatran. Hence patients who have this SNP (332G>A, 581C>T, 717C>A, 913C>T, 977C>T) would have decreased hCES1 function, which would result in therapeutic failure or sub-therapeutic drug action.

Keywords: Dabigatran etexilate, Carboxylesterase 1, Pharmacogenomics, Bioavailability, Genetic polymorphisms

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INTRODUCTION

Dabigatran etexilate (DABE) is a direct thrombin inhibitor primarily used in prophylaxis of stroke or venous thromboembolism in patients with valvular or non-valvular atrial fibrillation and atrial flutter [1]. It is an effective and relatively safe direct thrombin inhibitor with clinically significant inter-individual variability in anticoagulation response [2, 3]. Despite its low bioavailability, linear dose-response relationship, and selective and reversible thrombin inhibition have made DABE the preferred anticoagulant of choice for prophylaxis of stroke and venous thromboembolism [4]. DABE is a prodrug that depends on hepatic carboxylesterase 1 (*CES1*) and intestinal carboxylesterase 2 (*CES2*) activation through sequential metabolism [5]. Mutations and allelic variations in *CES1* gene have been reported to be a significant cause of variability in dabigatran-associated anticoagulation response. Polymorphisms in the coding exonic regions and non-coding promotor and/or untranslated regions (UTR) of CES1 gene have been reported in diverse populations. The human CES1 gene is relatively more polymorphic than CES2. Though the frequencies of CES1 genetic variations is less in Asian population, variations in the exonic and promotor regions of CES1 have been reported to significantly impair the DABE prodrug activation process [6]. Sub-therapeutic plasma concentrations of the active metabolite of DABE (Dabigatran) increase the risk of stroke and/or pulmonary embolism in patients with non-valvular atrial fibrillation and atrial flutter [7].

Several intronic, exonic and missense mutations have been reported to be associated with hepatic *CES1*and intestinal *CES2* as listed in table 1, respectively**.** Though the genotype frequencies of these polymorphisms in given definite populations are unclear, it is evident that variants of *CES1* can significantly alter DABE activation. Hence, it is crucial to explore the effect of genetic polymorphism on the pharmacokinetic and Pharmacodynamics (PK/PD) of DABE.

The effect of few genetic variations of *CES1* on DABE bioactivation has been studied and reported. While certain SNPs within the coding exonic regions tend to alter the protein conformation and stability and subsequently decrease the catalytic activity of *CES1*, few deletion and frameshift mutations tend to cause complete loss of catalytic activity due to early truncation and decrease in length of the peptide sequence [8, 9]. Therefore, it is evident that the bioactivation of DABE prodrug is critically impaired by the presence of allelic variants that are predominantly non-synonymous [10]. The effect of duplication of chromosomal loci bearing *CES1* on the extent of DABE bioactivation has not been studied so far, although duplication variations of *CES1* have also been reported. As DABE is a lipophilic molecule with dose-dependent pharmacokinetics, it could be theoretically assumed that chromosomal duplication of CES1 could increase the rate of bioactivation and precipitate the risk of visceral bleeding due to an increase in active dabigatran concentrations [11]. Pharmacokinetic investigations to study the effect of CES1 chromosomal duplications have not been reported. Moreover, variations in the transcription regulatory and promoter regions of *CES1*gene need to be explored to determine their effect on CES1 levels [12].

Apart from this genetic variations many other factors such as drug compliance, drug-drug interactions and drug-food interaction also play an important role in effecting the drug metabolism, which sometimes result in severe adverse drug reactions (ADR) sometimes fatal to the patients [13]. In recent years Bioinformatics tools are playing an important role in finding out the targeting sites of the drug and also the role of genetic variants on drug metabolism. These bioinformatics tools help in minimizing the experiment cost, increasing the accuracy and robustness of the studies [14]. The current study is aimed to demonstrate the effect of CES1 genetic polymorphisms on protein structure and dabigatran etexilate bioactivation through bioinformatics applications.

Genetic polymorphisms in human *CES1* gene

Table 1: Genetic polymorphisms in human hepatic *CES1* gene

Identifier	Chromosome	Functional consequence	Clinical significance
rs71647871	16:55823658	Missense	Drug-response [4]
rs121912777	16:55823661	Missense	Pathogenic [5]
rs1968753	16:55811439	Intron variant	Unknown [4, 6]
rs2244614	16:55810705	Intron variant	Unknown [4]
rs2302722	16:55811015	Intron variant	Unknown [7]
rs3815583	16:55833130	UTR variant 5 prime	Unknown [15]
rs4122238	16:55822805	Intron variant	Unknown [4, 5]
rs12149370	16:55833075	UTR variant 5 prime	Unknown [4]
rs34428341	16:55834169	Upstream variant 2KB	Unknown [5]
rs28563878	16:55833022	Missense	Unknown [6]

UTR: Untranslated region 5 Prime

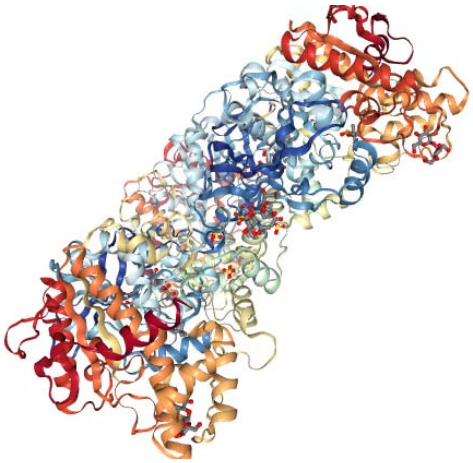

Fig. 1: Crystal structure of human *CES1* (PDB Id: 2HRQ)

MATERIALS AND METHODS

Target modeling

Three-dimensional structure of Human Carboxyl esterase 1 (hCES1) was obtained from the protein data bank (PDB). The primary sequence of wild type variant which was retrieved showed the presence of the following residues: Serine75, Alanine158, Aspartate203, alanine269 and Threonine290 Using Swiss-PDB viewer the former variant was mutated and modeled with following changes Asparagine75, Valine158, Glutamate203, Serine269 and Methioinine290. Targets were prepared by removal of water, addition of polar hydrogen's and gasteiger charges.

Energy minimization

Target protein structures were subjected to energy minimization by using YASARA server that empirical energy functions called 'knowledge-based potentials' to explain force calculations. (http://www.yasara.org/minimizationserver.htm).

Protein conformation and stability

Dihedral angles (phi and psi) and bond order of the energy minimized proteins were analyzed through Ramachandran Plot. Swiss-PDB viewer was used to create the Ramachandran plot.

Active site prediction

The drug-binding pocket of hCES1 was determined by the Prankweb server, which employs a local chemical neighborhood ligandability to compute P2 rank. (http://prankweb.cz/).

Molecular docking analysis

We performed molecular docking analysis using Autodock 4.2.6. The catalytic trial and active site of Human Carboxylesterase 1 (hCES1) was pre-defined by a three-dimensional grid using the Auto grid program. Twenty-seven thousand conformers were generated in 25000 evaluations by genetic algorithm. Target-ligand complexes were visualized using Pymol 2.3 (Schrodinger, LLC). Ligand interactions with the active site residues were analyzed by 2D maps constructed using LeView software.

RESULTS AND DISCUSSION

The Human Carboxylesterase 1 (hCES1) is a hepatic hydrolase enzyme and serves in hepatocellular bio activation and metabolism of several xenobiotics, including oral anticoagulant Dabigatran etexilate and various endogenous substances. Missense mutations in the human CES1 gene are linked with abolishment of glycosylation and loss ability to metabolize dabigatran etexilate. Hence, we analyzed the impact on the metabolism of Dabigatran etexilate due to Non-synonymous polymorphism (332G>A, 581C>T, 717C>A, 913C>T, 977C>T) by *in silico* approaches. Native biological conformation of the modeled target proteins was achieved through energy minimization. The potential energies of the target proteins before and after energy minimization are shown in table 2.

Potential energies of modeled hCES1 enzyme variants

Protein conformation and energy-minimized target stability were analyzed by the Ramachandran plot by dihedral angles and atomic contacts. Ninety-eight percent of the residues occurred within the allowed region of the plot for all variants explaining the least steric interactions and reliable spatial geometry for the modeled structures. The docking results were depicted in table 2.

Table 2: Pre and post-minimization potential energies of the wild-type and mutant variants

S. No.	Type of variant	Pre-minimization potential energy	Post-minimization potential energy
1	Wild Type	28895.3058 kcal/mol	-927 kcal/mol
2	Ala158Val	34411 kcal/mol	-127.2448 kcal/mol
3	Ser75Asn	28745.2540 kcal/mol	-540.2540 kcal/mol
4	Asp203Glu	11268.846 kcal/mol	-354.22 kcal/mol
5	Ala269Ser	33854.69 kcal/mol	-228.82 kcal/mol
6	Thr290Met	8399.2681 kcal/mol	-732.238 kcal/mol

The effect of single nucleotide variations on the catalytic triad and active site of hCES1 was determined by visual analysis of active site residues. Thus, it is evident that single nucleotide variations in hCES1 impair the bio-activation dabigatran etexilate by disrupting

the active site and catalytic triad. Alterations in DABE interactions with the catalytic triad and active site residues provoked by conformational changes were investigated through molecular docking analysis. Ligand interaction and binding conformations were analyzed in terms of the following parameters: Binding energy (ΔG Kcal/mol), inhibitory constant (kI), conformational orientation of the ligand in the active site, hydrogen bonding, π-π interactions and root mean square deviation (RMSD)(Morris *et al.*, 1998). The Molecular Docking analysis of Dabigatran with Wild type, Ser75Asn, Ala158Val, Asp203Glu, Ala269Ser and Thr290Met shown in table 3

Hydrogen bonding interactions with the active site residues of Wild type, Ser75Asn, Ala158Val, Asp203Glu, Ala269Ser and Thr290Metare shown in table 3.

Table 3: Molecular docking analysis of dabigatran

Variant type	ΔG (Kcal/mol)	KI	Hydrogen bonding	Vanderwaal's interaction
Wild	-7.21	0.32	ALA 158, SER221	HIS468, GLU354
Ser75Asn	-3.20	1.58	SER221	-
Ala158Val	-1.57	3.46	-	-
Asp203Glu	-2.45	2.38	-	HIS 468
Ala269Ser	-3.38	1.69	-	-
Thr290Met	-4.11	1.18	SER221	GLU354

The Three Dimensional Docked conformation of Dabigatran with the Wild type, Ser75Asn, Ala158Val, Asp203Glu, Ala269Ser and Thr290Met are shown in fig. 2-2a, 2b, 2c, 2d, 2e and 2f Represent Wild Type, Ser75Asn, Ala158Val, Asp203Glu, Ala269Ser and Thr290Met Variants Respectively

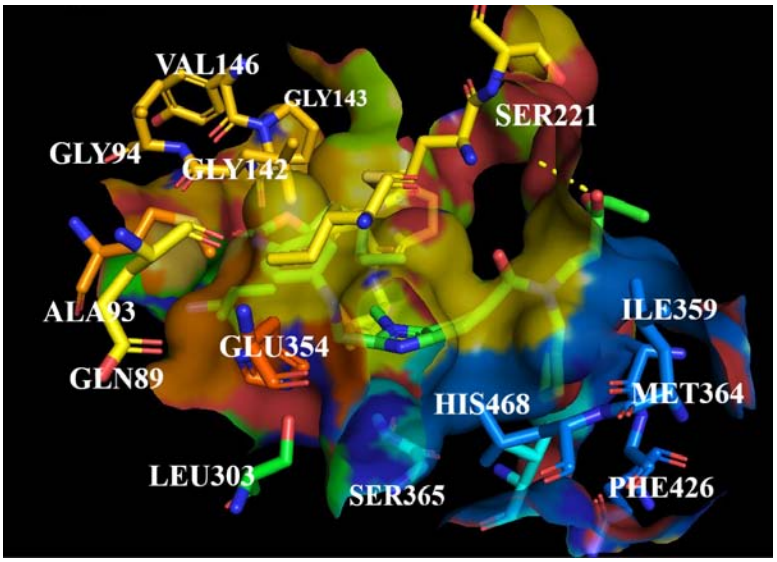

Fig. 2a: Wild-type variant

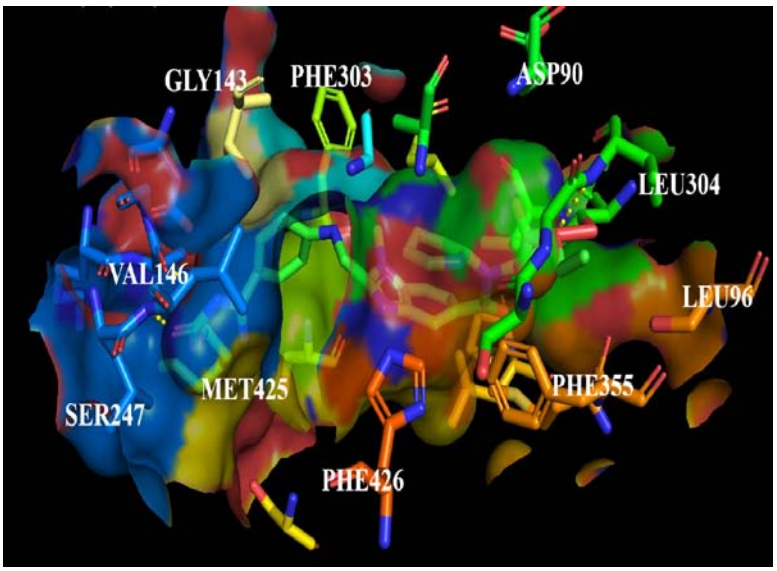

Fig. 2b: Ser75Asn variant

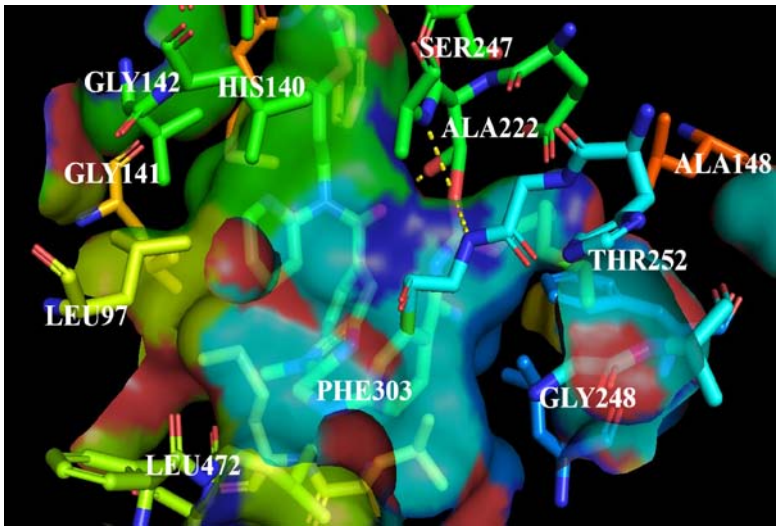

Fig. 2c: Ala158Val variant

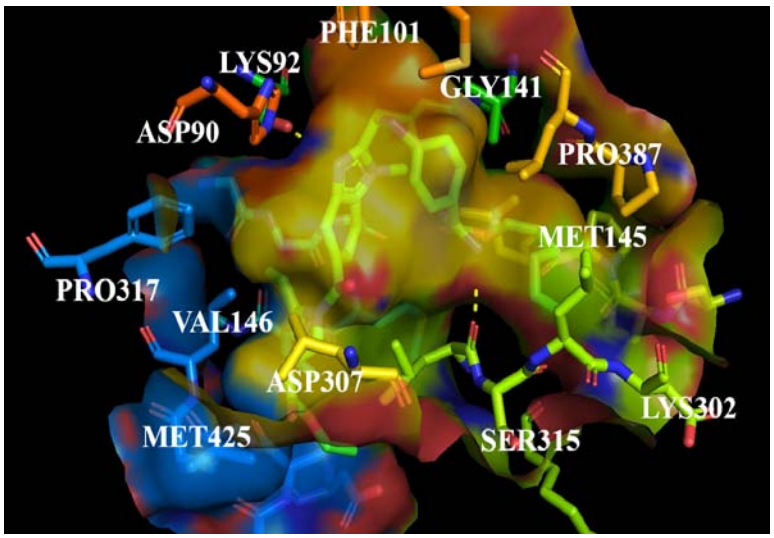

Fig. 2d: Asp203Glu variant

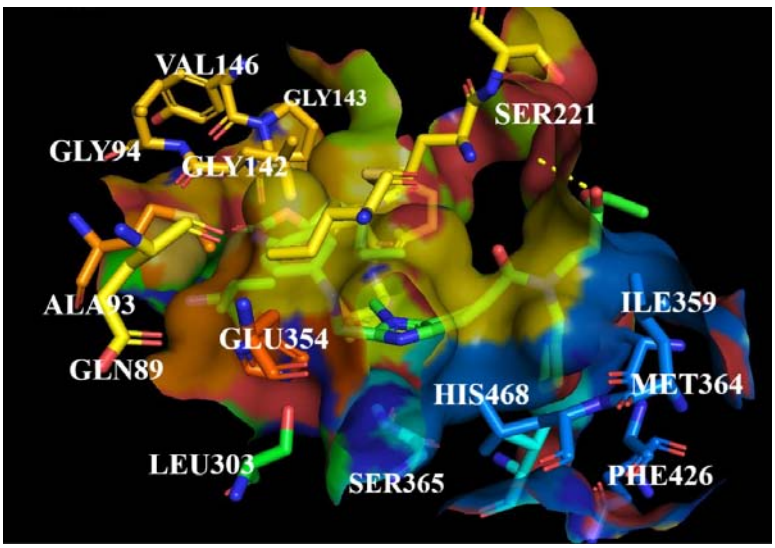

Fig. 2e: Ala269Ser variant

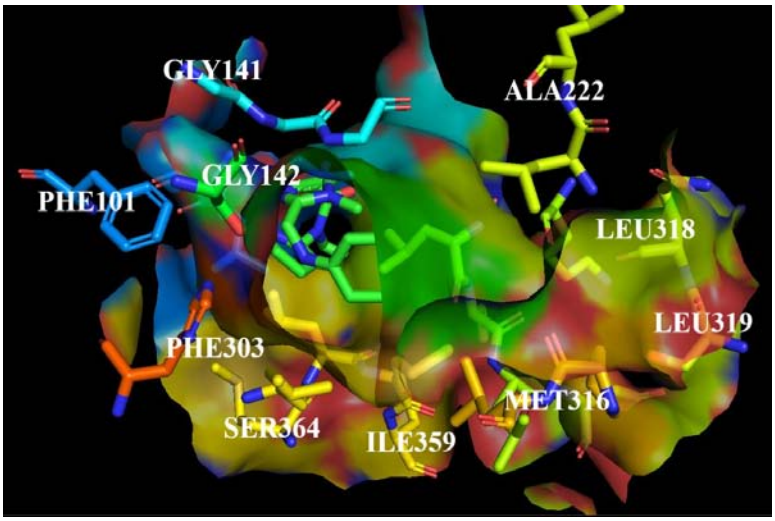

Fig. 2f: Thr290Met variant

DISCUSSION

Human carboxyl esterase (hCES1) is a serine esterase containing the characteristic α/β esterase fold and a wide ligand binding cavity with rigid and flexible active sites [16]. In order to ensure realistic conformations of wild and modeled mutant structures and assess their conformation stability, we analyzed bond geometry using Ramachandran Plots. We visualized dihedral angles phi and psi and observed no residues in strictly forbidden regions of the plot of either wild-type or mutant structures. While 99.5% residues occurred within the favored region of the plot of wild type, Ser75Asn, Ala158Val and Thr290Met variants, with exceptions being glycine and proline, 98% residues occurred within the favored of the plot of Asp203Glu and Ala269Ser variants and the remaining in the outlier region. We observed bond geometries of wild variant of *hCES1* much similar to that reported previously [17, 18].

We used PrankWeb to identify drug binding pockets. PrankWeb is a template-free, P2Rank-based server that employs random decision forests to predict the ligand binding domains on protein topology [19]. The catalytic triplet of the hCES1 active site made of Serine 221, Histidine 468 and Glutamic acid 354 is neighbored predominantly by hydrophobic amino acids. We also observed that hydrophobic amino acids predominantly formed the active site of hCES1 in all our variants. However, a significant difference in residues forming the active site was observed between each of our variant in the energy minimized structures. Such differences were insignificant pre-minimization, suggestive of the proper molecular and bond arrangement achieved post-energy minimization using YASARA force fields. In addition, the catalytic triad itself was either partially absent in Asp203Glu and Thr290Met variants while it was completely absent in Ser75Asn, Ala158Val and Ala269Ser variant. These post-energy minimization findings are suggestive that the studied non-synonymous variations significantly alter the conformation of the hCES1 catalytic domain. YASARA server. YASARA force fields use the Boltzmann's equation to calculate empirical energy functions called 'knowledge-based potentials' to explain force calculations [20]. Missense mutations in hCES1 are reported to affect hCES1 function and expression, thereby altering substrate metabolism [21]. Non-synonymous polymorphisms in hCES1 gene cause amino acid changes that can significantly disrupt potential drug binding sites and the catalytic triad and can ablate the catalytic potential of the enzyme [22]. Serum concentration of prodrug substrates of hCES1, such as dabigatran etexilate can hence be sub-therapeutic in carriers of non-synonymous variations, including Ser75Asn, Ala158Val and Ala269Ser [6]. The mutation of Ser75Asn will result in inter-molecular interactions seen in CES 1 due to the interaction of Ser75 and Arg186 [23]. According to a previous population pharmacokinetics study, there was an increase in d-MPH (Methylphenidate) AUC by 68.21% due to TG SNP in exon 7. A study depicts that C>A SNP in intron 10 was correlated with a substantial 15% reduction inactive dabigatran etexilate per minor allele plasma trough concentrations. This pharmacokinetic effect was further accompanied by a significant reduction in the risk of bleeding with an OR of 0.67 per minor allele [24]. Treatment failure with dabigatran etexilate can increase the risk of adverse cardiovascular outcomes, including thromboembolism [25]. Hence, it is crucial to determine hCES1 status in patients with non-valvular atrial fibrillation who are about to be started on dabigatran etexilate.

CONCLUSION

The mutant binding energy (79Ala, 221Ala, 354Ala and 468Ala) of Dabigatran was found to be less than that of the wild form. This suggests that the presence in hCES1 of functional non-synonymous polymorphism greatly alters Dabigatran binding. It also helps to collect data regarding the drug's pharmacokinetics and pharmacodynamics. Therefore, patients with this SNP (332G>A, 581C>T, 717C>A, 913C>T, 977C>T) would have reduced hCES1 activity, resulting in therapeutic or sub-therapeutic drug response failure. It also provides patients who have such SNPs with a tailored treatment approach.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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