BMC Pharmacology and Toxicology

RESEARCH

Open Access

A comprehensive insight from molecular docking and dynamics with clinical investigation on the impact of direct oral anticoagulants on atheroprotective protein in atrial fibrillation

M. Sudhan[1], V. Janakiraman[1], Sheikh F. Ahmad[2], Sabry M. Attia[2], Ramasamy Subramanian[3], Durga Devi[4] and Shiek S. S. J. Ahmed[1*]

Abstract

Background Direct oral anticoagulants (DOACs) have high potency against their therapeutic target and are widely used in the treatment of atrial fibrillation (AF). Most DOACs are often claimed to have adverse effects due to off-target inhibition of essential proteins. Human serum paraoxonase 1 (PON1), one of the essential proteins, known for its anti-inflammatory and antioxidant properties, could be affected by DOACs. Thus, a comparative evaluation of DOACs and their effect on PON1 protein will aid in recommending the most effective DOACs for AF treatment. This study aimed to assess the impact of DOACs on PON1 through a combination of computational and experimental analyses.

Methods We focus on apixaban, dabigatran, and rivaroxaban, the most recommended DOACs in AF treatment, for their impact on PON1 through molecular docking and molecular dynamics (MD) simulation to elucidate the binding affinity and drug-protein structural stability. This investigation revealed the most influential DOACs on the PON1 protein. Then experimental validation was performed in DOAC-treated AF participants ($n=42$; 19 treated with dabigatran and 23 treated with rivaroxaban) compared to a healthy control group ($n=22$) through gene expression analysis in peripheral blood mononuclear cells (PBMC) and serum enzyme concentration.

Results Our computational investigation showed rivaroxaban (−4.24 kcal/mol) exhibited a lower affinity against the PON1 protein compared to apixaban (−5.97 kcal/mol) and dabigatran (−9.03 kcal/mol) through molecular docking. Dabigatran holds complex interactions with PON1 at GLU53, TYR197, SER193, and ASP269 by forming hydrogen bonds. Additionally, MD simulation revealed that dabigatran disrupts PON1 stability, which may contribute functional changes. Further experimental validation revealed a significant down-regulation ($p<0.05$) of *PON1* gene expression in PBMC and decreased serum PON1 enzyme concentration on DOAC treatment. Rivaroxaban as about 48% has inhibitory percentage and dabigatran as about 75% of inhibitory percentage compared to healthy control.

*Correspondence:
Shiek S. S. J. Ahmed
shiekssjahmed@gmail.com

Full list of author information is available at the end of the article

Conclusion Overall, our computational and experimental results clearly show the higher inhibitory effect of dabigatran than rivaroxaban. Hence, rivaroxaban will be a better drug candidate for improving the outcome of AF.

Keywords Atrial fibrillation, PON1, MD simulation, Enzyme level, Gene expression

Background

Coronary artery disease (CAD) is one of the leading causes of mortality and morbidity worldwide. The pathogenesis related to CAD was a sequential event initiated by atherosclerosis, myocardial infarction, atrial fibrillation (AF), and heart failure [1]. AF is one of the critical events that cause an irregular and often very rapid heart rhythm (arrhythmia) that can lead to blood clots in the heart, leading to mortality that accounts for 33 million deaths worldwide [2]. AF has a lifetime risk of 1–2% in the general population and is linked to consequences such as stroke, heart failure, and death [3]. Risk factors for AF include age, gender, genetics, diabetes mellitus, cholesterol (low-density lipoproteins (LDL) and high density lipoproteins (HDL)), hypertension, obstructive sleep apnea, metabolic syndrome, and smoking, all of which play a critical role in AF pathogenesis [4]. Among these, genetic factors were often defined as important risk factors for AF [5]. In the absence of a solid scientific foundation, there is considerable practice variation in the selection of treatment/drug for managing AF during severe illness [6]. Currently, several drugs have been recommended for AF treatment. These drugs interact with multiple proteins in the human system to treat sustained arrhythmia. Recently, the use of direct oral anticoagulant drugs (DOACs) for the treatment of non-valvular AF has increased compared to vitamin K antagonists (due to their various limitations) [7]. Moreover, DOCAs have been shown to prevent thromboembolic events and are safe, having a lower bleeding risk than warfarin [8]. Of several recommended drugs, DOACs such as apixaban, dabigatran, and rivaroxaban are most popular to control or prevent thrombotic events in AF [9]. Alternatively, a few of these DOACs have significant off-targets that are capable of inhibiting the beneficial proteins, which decreases the treatment efficiency and causes side effects [10].

Human paraoxonase 1 (PON1) is a multi-functional protein encoded by the PON1 gene, which is localized at chromosome 7q21.3. PON1 consists of nine exons that synthesize protein with 355 amino acids [11]. PON1 is extensively expressed in the liver and helps to detoxify foreign toxins, such as pesticides, which are closely associated with a person's vulnerability to harmful compounds [12]. Additionally, PON1 is involved in lipid peroxidation, innate immunity, oxidative damage, cell proliferation, modulation of endoplasmic reticulum stress, and apoptosis [13]. Recently, PON1 has been recognized as one of the key antioxidants that protects against the oxidation of LDL and HDL [14]. Interestingly, the expression of PON1 helps to eliminate cholesterol, which helps to prevent thrombotic events that are critically associated with CAD, stroke, and AF [15]. Argan et al. demonstrated that few cardiovascular drugs have the ability to inhibit the human PON1 protein [16]. However, such an inhibitory effect was not examined in the AF patients in association with PON1 target. Hence, establishing the effect of DOACs against PON1 in AF patients will guide the recommendation of an effective drug for potential treatment.

In this study, we investigated the most widely used DOACs (apixaban, dabigatran, and rivaroxaban) against PON1 through computational and experimental approaches. Initially, the PON1 structure was modeled and docked with the DOACs. Then, the DOACs that showed lower and higher glide scores in docking were evaluated for their structural stability through molecular dynamics (MD) simulation. Further, the expression of PON1 was detected in participants (control and test groups) by collecting blood samples from AF individual under selective DOAC treatment. Additionally, the PON1 enzyme level was assessed in serum to check whether the DOACs affected the PON1 concentration. Overall, our analysis provides valuable insights into the off-target effect of DOACs against PON1 protein, with the candidate drug that might help AF patients efficiently.

Materials and methods

Retrieval of protein sequence, modeling, and validation

The PON1 sequence was retrieved from the UniProt database (UniProt ID: P27169). Then, the retrieved sequence was used to search for its protein structure in the protein data bank (PDB). Due to the unavailability of a complete PON1 structure, homology modeling was performed using the Swiss-model server (https://swiss-model.expasy.org/) to generate structure [17]. Further, the modeled PON1 protein structure quality was verified using PROCHECK, VERIFY3D, and ERRAT tools in the SAVES server (https://saves.mbi.ucla.edu/). The refined best structure was then energy optimized and used for molecular docking.

Retrieval of anticoagulant drugs

Simultaneously, the three dimensional chemical structures of DOACs, namely apixaban (CID: 10182969), dabigatran (CID: 216210), and rivaroxaban (CID: 9875401), were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The structures of these DOACs

were downloaded in structure data file (SDF) format and optimized before molecular docking.

Structure optimization and molecular docking

All collected drug structures were imported into the LigPrep module, Schrödinger (https://www.schrodinger.com/products/ligprep) and optimized with the OPLS force field. Meanwhile, the modeled PON1 protein structure was optimized with OPLS force field through the protein preparation wizard, Schrödinger. Both protein and DOACs were prepared for subsequent molecular docking investigation. The Schrödinger suite's Glide module was utilized to dock each DOAC to the PON1 protein at their active site identified using a prankweb server (https://prankweb.cz/). Finally, the DOACs showing extreme high and low affinity to PON1 were selected and subjected to MD simulation.

Molecular dynamic (MD) simulation

The MD simulation was conducted by GROningen Machine for Chemical Simulation 2021.6 (GROMACS 2021.6) [18]. The protein topologies were prepared using the CHARMM36 all-atom force field [19]. Likewise, the ligand topology for selected DOACs was generated using the CGenFF 2.4.0 version (CGenFF Home (umaryland.edu)). The protein-drug complex was positioned in a dodecahedron box at least 1 Å from the edges of the box and filled with TIP3P water molecules [19]. Then, the system was neutralized with Na+ ions and the particle mesh Ewald (PME) approach [20]. The steepest descent approach was used to minimize the simulation system by 50,000 steps. The temperature and pressure were set at 300 K and 1.0 bar using constant Number of particles, volume, and temperature (NVT) and constant Number of particles, pressure and temprature (NPT) ensembles, respectively. Parrinello-Rahman barostat was used to maintain the pressure [21]. The linear constraint solver method was implemented for long-range electrostatic interactions with periodic boundary conditions [22]. The MD simulation was carried out for 100 ns, and their trajectory coordinates were saved at every 1 ps. Further, MD trajectories such as backbone root mean square deviation (RMSD), protein root mean square fluctuation (RMSF), backbone solvent accessible surface area (SASA), backbone radius of gyration (Rg), and protein hydrogen bond (HB) were assessed for the protein dynamics and plotted using XM Grace Software (http://plasma-gate.weizmann.ac.il/Grace/).

Sample collection

The cohort of 22 healthy individuals (control) and 42 AF samples (rivaroxaban = 23; dabigatran = 19) were collected for this study. AF participants were recruited from the Department of Cardiology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Tamil Nadu, India, between March and November 2022. The recruitment of participants was based on the inclusion and exclusion criteria. In the inclusion criteria: all AF participants who were diagnosed and were under DOACs, either rivaroxaban or dabigatran, for a minimum of three months were selected. Similarly, the healthy control samples were confirmed based on the master health check-up and clinical examination by the cardiologist. All recruited participants were of South Indian origin and aged between 43 and 67 years of age, male and female. Alternatively, the exclusion criteria include a control participant under any inflammatory or antioxidant treatment. Similarly, among AF participants under multiple AF drugs, individuals with smoking and/or alcoholic habits were also excluded. This present study follows the Helsinki Declaration, and the study design was approved by the Human Ethics Committee (IHEC-II/0118/21) of the Chettinad Academy of Research and Education. A signed informed consent form was obtained in local language from the participants prior to the sample collection. In addition, a systematic questionnaire was used to elicit the general and clinical characteristics of each participant.

Demographic and clinical data

Using the systematic questionnaire, both demographic and clinical data were collected from control and AF. The data includes age, gender, body mass index (BMI), total cholesterol (TC), riglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and haemoglobin A1c (HbA1c) as a part of clinical investigation.

PON1 gene expression analysis

Peripheral fasting blood (4 ml) was collected from both control ($n=22$) and AF participants ($n=42$) in a vacutainer tube. Exactly 3 ml of ethylenediaminetetraacetic acid (EDTA) containing blood was used to isolate the PBMC using Histopaque-1077 solution following the manufacturer's protocol (Sigma-Aldrich). Next, the total RNA was extracted using the RNAiso Plus (Takara Total RNA Extraction Reagent) and quantified using a Nanodrop spectrophotometer (Thermo Scientific, USA). A total of 100 ng of RNA was used as a template to construct the cDNA with the reverse transcriptase core kit (Eurogentec, Senaing, Belgium). Then gene expression was assessed using the Real-Time qPCR System (Applied Biosystems QuantStudio 5) with gene-specific primers (forward: TTTCACCCGATGGCAAGTATG and reverse: TCTTATGAGCCAGCAACTCAGC) of *PON1* and *GAPDH* (forward: AAGGTGAAGGTCGGAGTCAA and reverse: ACATGTAAACCATGTAGTTGAGGT) as references. Finally, the PON1 expression was quantified

in the control and AF-treated (rivaroxaban and dabigatran) groups following the $2^{-\Delta\Delta Ct}$ method.

PON1 concentration in blood serum

The remaining 1 ml of blood was used to isolate serum and assessed for PON1 enzyme concentration through a pre-coated enzyme-linked immunosorbent assay (ELISA) microplate. The serum sample from each group was processed to determine enzyme concentration using the Paraoxonase assay kit (Elabscience's ELISA kit: E-EL-H2298) by following the manufacturer's protocol. Briefly, the samples are introduced into each well, where they interact with the specific antibody that has been pre-coated on the plate. The plate was then incubated with a biotinylated detection antibody that is specific for Human PON1 and an Avidin-Horseradish Peroxidase (HRP) conjugate. A substrate solution is introduced to each well after any unbound components were washed away during incubation. Finally, the reaction was stopped by the addition of a stop solution, which results in a colour formation that was quantified at a wavelength of 450 nm using the microplate reader. Further, using the generated standard plot, the levels of PON1 enzyme were determined.

Statistical analysis

All statistical analysis was performed using Graphpad Prism software. The demographic and clinical data were represented as the average ± standard deviation. Also, the differences in PON1 gene expression and enzyme concentration between the groups were presented as a bar graph, and their statistical significance ($P<0.05$) was assessed through the analysis of ANOVA with a post hoc Tukey's test for comparative analysis. Further, the percentage of relative inhibition of serum PON1 was calculated using the formula below.

$$Relative\ Suppression\ (\%) = \frac{PON1 average concetration of control - PON1\ average\ concetration\ of\ DOAC}{PON1\ average\ concetration\ of\ control} \times 100$$

Results

Protein modeling and validation

The three-dimensional structure of PON1 was modeled using the Swiss model. We found more than 50 templates relevant to the given PON1 protein sequence. Of these, PDB ID: 6H0A was selected as an appropriate template for modeling the PON1 structure, which showed a GMQE score of 0.93 with the restriction of only one gap. The sequence homology between PON1 with the selected template was 82.25%, which was higher than the required 30% for modeling protein structures. Using the template, the structure was modeled and verified for its quality based on ERRAT, VERIFY3D, and PROCHECK. Generally, a quality score of 80 % or above in ERRAT is considered to be a good model. Our modeled PON1 has an ERRAT quality of 92.53%, which was higher than the required percentage. Likewise, the predicted PON1 model was validated by VERIFY3D and PROCHECK servers. The VERIFY3D showed a good quality score of 83.48%, and PROCHECK showed 86.4% of amino acids were noticed in the allowed region, respectively. Overall, the generated PON1 structure (Fig. 1A) was of high quality and suitable for subsequent analysis.

Molecular docking of DOACs with PON1

The three DOAC (apixaban, dabigatran, and rivaroxaban) structures were collected, optimized, and docked with PON1 structure by using the Glide docking module in Schrödinger software. The glide score energies of apixaban, dabigatran, and rivaroxaban with PON1 were –5.97, –9.03, and –4.24 kcal/mol, respectively. Interestingly, the lowest affinity was noticed for rivaroxaban (–4.24 kcal/mol) and the highest affinity was observed with dabigatran (–9.03 kcal/mol), which forms significant interaction around the active sites (GLU53, PRO72, ILE74, HIS115, HIS134, ASN168, PHE222, ASN224, ASP269, ILE291, and PHE292) of the PON1 protein (Fig. 1B–D). Notably, apixaban and PON1 (PHE292) formed a Pi-Pi stacking. Whereas dabigatran interacted with the PON1 at GLU53, TYR197, SER193, and ASP269 residues by forming hydrogen bonds, while Pi-Pi stacking with the TYR71, PHE222, and PHE292 residues. However, rivaroxaban formed two hydrogen bonds with the PON1 amino acid SER193. For comparison, the DOACs presenting lowest (rivaroxaban) and highest (dabigatran) affinity were selected and used for MD simulation to understand the structural changes in PON1 protein upon binding of the DOACs.

Molecular dynamics simulation

The structural stability of PON1 with the selected DOACs (rivaroxaban and dabigatran) was determined based on the backbone RMSD, protein RMSF, backbone SASA, backbone Rg, and protein HB trajectories through MD simulation. The average RMSD, RMSF, SASA, Rg, and HB of apo-PON1 (ligand free PON1) were 0.217 nm, 0.118 nm, 177.347 nm^2, 1.881 nm, and 238, respectively. Likewise, the PON1 with rivaroxaban shows an average trajectory of 0.250 nm, 0.130 nm, 179.297 nm^2, 1.894 nm, and 238 for RMSD, RMSF, SASA, Rg, and HB, respectively. Whereas the average RMSD, RMSF, SASA, Rg, and HB trajectories for dabigatran with PON1 were 0.210 nm, 0.133 nm, 179.294 nm^2, 1.893 nm, and 233. Finally, the differences in trajectories were assessed by

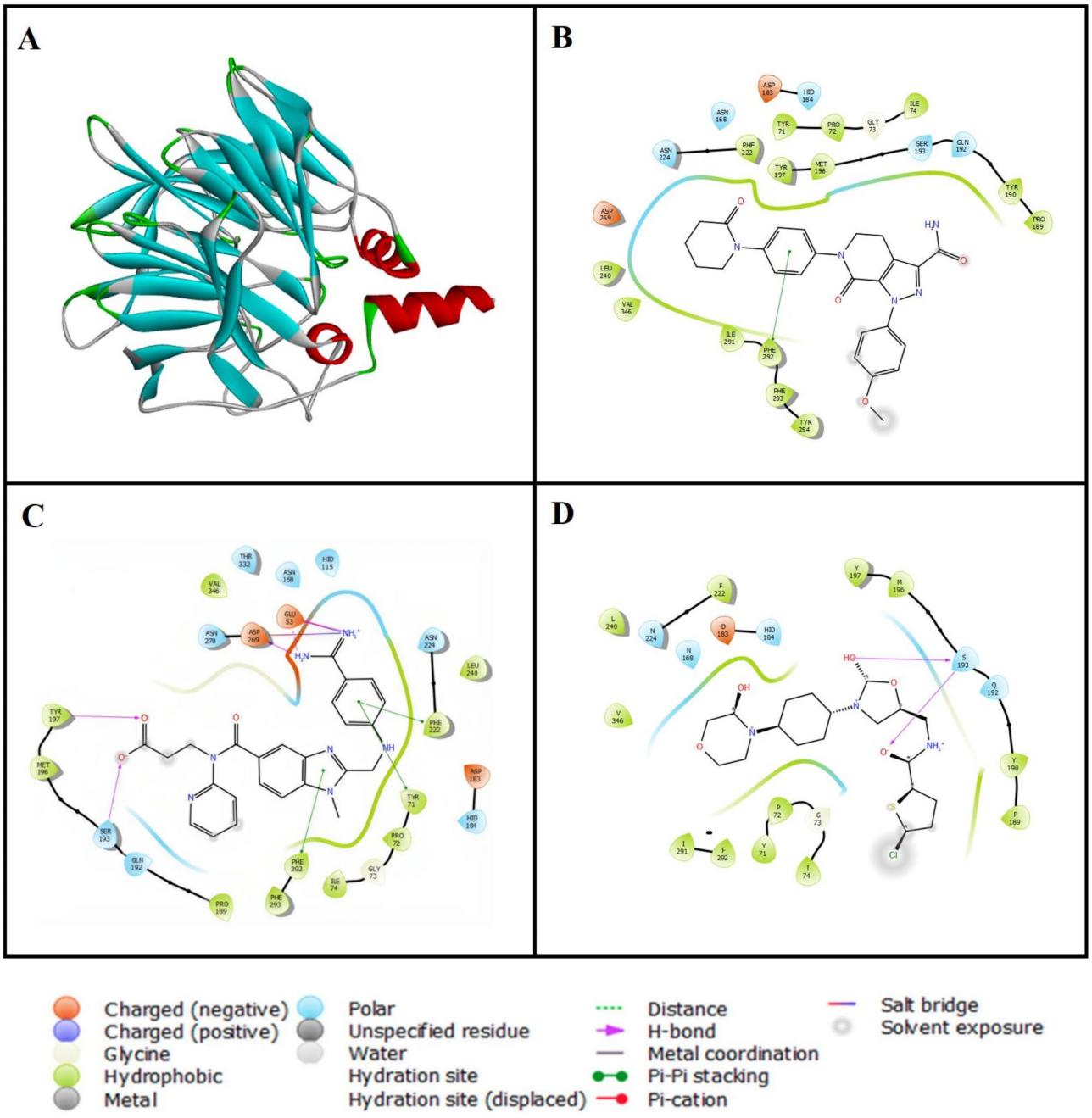

Fig. 1 Binding interaction of protein and direct oral anticoagulants. **A**) Predicted PON1 protein structure, **B**) Two dimensional interactions of PON1 with apixaban, **C**) Two dimensional interactions of PON1 with dabigatran, and **D**) Two dimensional interactions of PON1 with rivaroxaban

generating the plot with the rivaroxaban and dabigatran-PON1 complexes in comparison with apo-PON1. The trajectory plots of RMSD, RMSF, SASA, Rg, and HB were illustrated in Figs. 2, 3 and 4. According to the simulation plot in Fig. 4A, the dabigatran complex with PON1 has become more stable than the apo-PON1. Whereas, the rivaroxaban complex has lower stability compared to apo-PON1, which suggests that rivaroxaban may have less inhibitory effect than dabigatran. Further, the parallel simulation was performed to check the reliability of MD simulation and their results were provided in the "Supplementary File 1".

Clinical characteristics and relative mRNA expression

The blood was collected from the AF (n=42) as well as the healthy controls (n=22), and their demographic characteristics were recorded and statistically analysed (Table 1). Notably, BMI, TC, TG, and LDL were

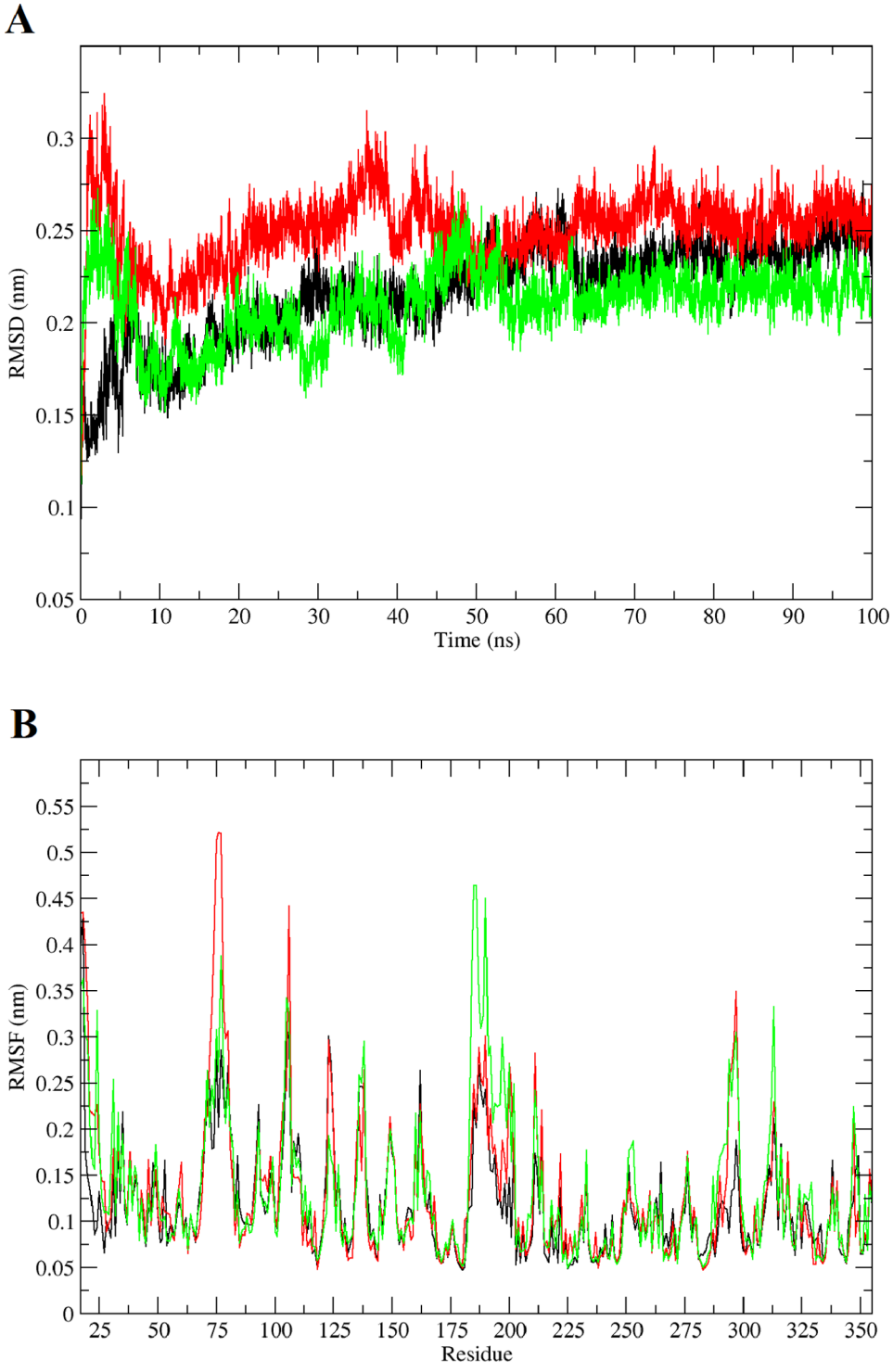

Fig. 2 Molecular dynamics simulation trajectories. Root mean square deviation (**A**) and root mean square fluctuation (**B**) of apo-PON1 (Black) and complexes (rivaroxaban (Red) and dabigatran (Green) for the time scale of 100 ns

increased, and HDL was decreased significantly in AF patients compared to healthy controls. Subsequently, the *PON1* gene expression was performed in each participant as mentioned in the methodology section. Based on the gene expression, the analysis showed significant down-regulation of *PON1* in AF participants when compared to control group. Particularly, AF participants under dabigatran had a lower level of expression than AF participants treated with rivaroxaban (Fig. 5A).

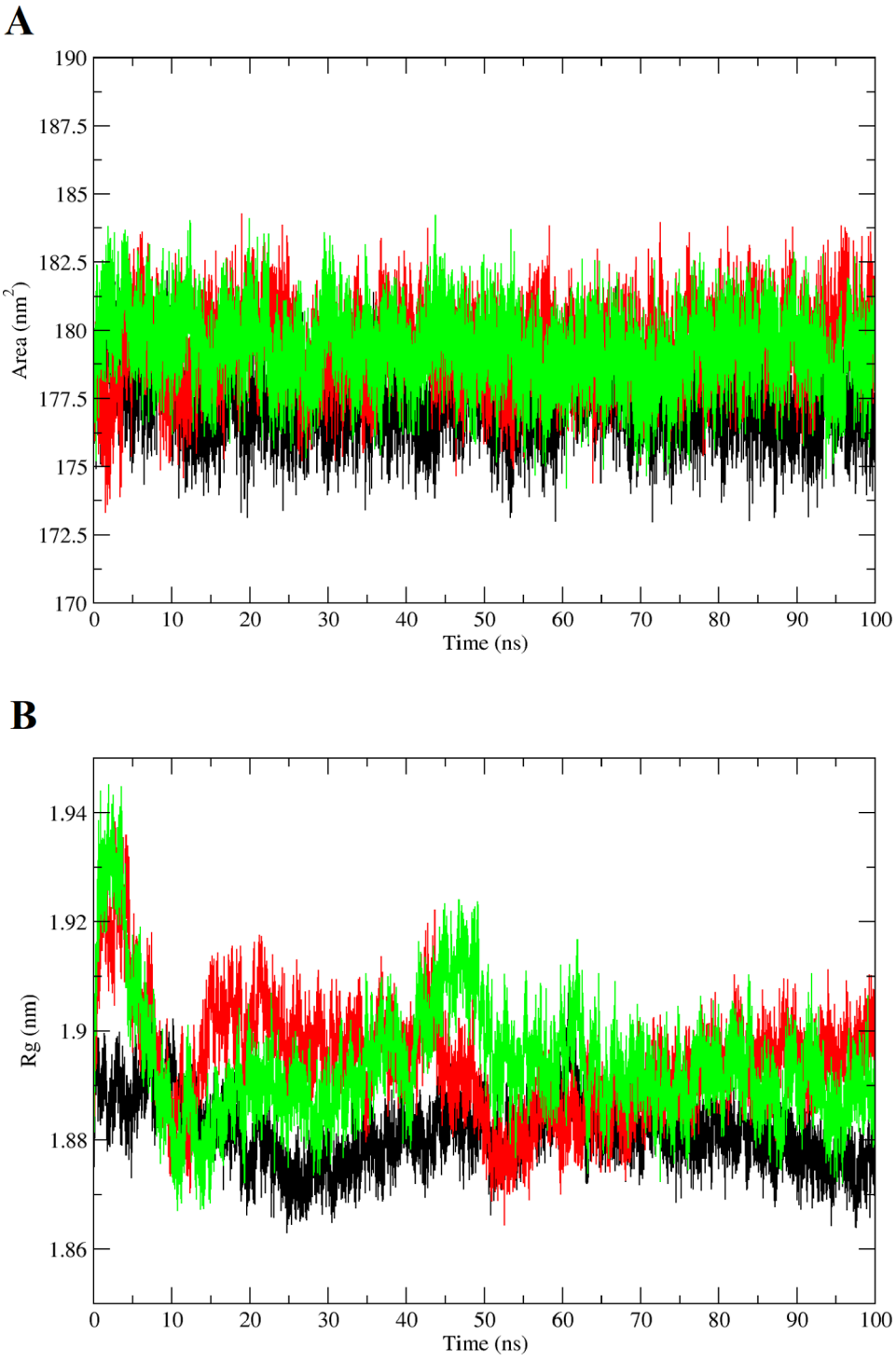

Fig. 3 Molecular dynamics simulation trajectories. Solvent accessible surface area (**A**) and radius of gyration (**B**) of apo-PON1 (Black), PON1 with rivaroxaban (Red), and PON1 with dabigatran (Green)

Serum PON1 enzyme concentration between the groups

PON1 concentration was assessed through the ELISA method in the blood serum of normal (n=22) and AF (n=42). A significant change in PON1 concentration was observed in DOAC-treated groups compared to the control group. Notably, AF individuals treated with rivaroxaban showed a marginal decrease in enzyme concentration compared with healthy controls (Fig. 5B). A similar trend was observed in dabigatran-treated AF participants when compared to healthy controls. In comparison

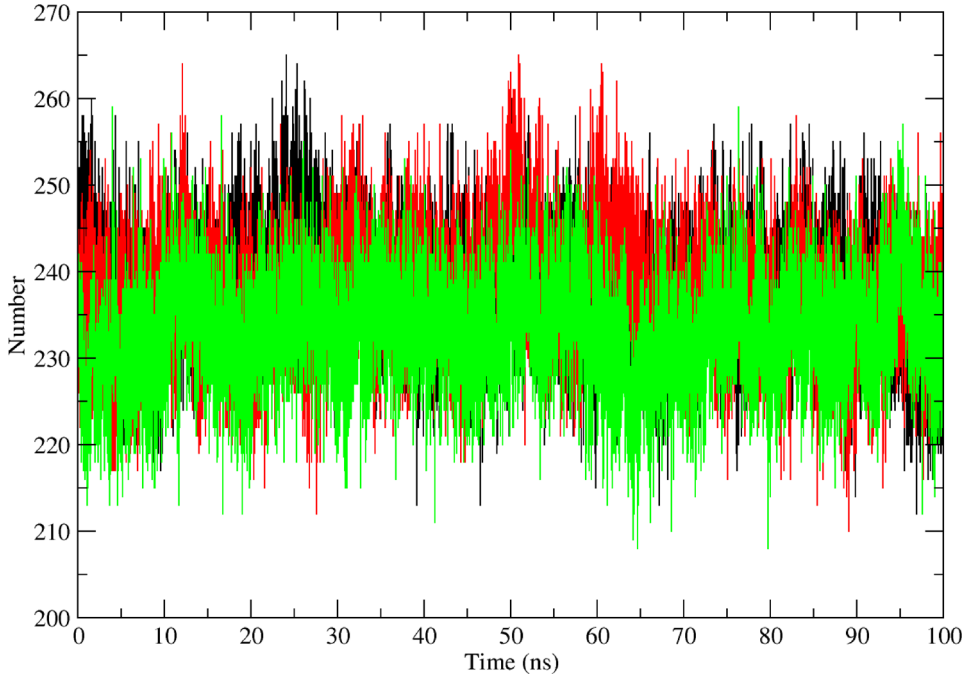

Fig. 4 Molecular dynamics simulation trajectories. Hydrogen bonds of apo-PON1 (Black) and the complexes (rivaroxaban with PON1 (Red) and dabigatran with PON1 (Green)) structures

Table 1 Demographic and clinical characteristics of the study participants

Characteristics	Controls (Average ± SD)	AF treated with rivaroxaban (Average ± SD)	AF treated with Dabigatran (Average ± SD)	*P* value (Control VS. rivaroxaban)	*P* value (Control VS. Dabigatran)
Samples size (Male/Female)	22 (M:12, F:10)	23 (M:12 F:11)	19 (M:10, F:9)		
Age (Year)	59 ± 3	61 ± 4	63 ± 2	$P > 0.05$	$P > 0.05$
BMI (kg/m^2)	22.60 ± 1.76	21.82 ± 1.09	21.38 ± 0.86	$P > 0.05$	$P > 0.05$
TG (mg/dl)	95.19	148 ± 12.43	193.82 ± 10.8	**$P < 0.001$**	**$P < 0.001$**
TC (mg/dl)	138.03 ± 15.31	194.34 ± 12	231.43 ± 18.45	**$P < 0.001$**	**$P < 0.001$**
LDL (mg/dl)	86.14 ± 3.4	121 ± 11.6	148.23 ± 16.54	**$P < 0.05$**	**$P < 0.05$**
HDL (mg/dl)	42.78 ± 3.5	35.08 ± 2.54	28.65 ± 1.6	**$P < 0.05$**	**$P < 0.01$**
HbA1c %	5.38 ± 1.2	5.42 ± 0.61	5.53 ± 1.32	$P > 0.05$	$P > 0.05$

$P > 0.05$: Insignificant; $P < 0.05$ denote significant

Bold characters represents the statistical significance ($P<0.05$) between DOAC treated (Dabigatran or Rivaroxaban) compared to healthy controls

between the DOAC-treated groups, the participants under dabigatran showed significantly ($p<0.05$) decreased PON1 concentration than the participants under rivaroxaban (Fig. 5B). Particularly, the relative suppression of serum PON1 in AF patients treated with rivaroxaban was 48.07 ± 4.53%, while dabigatran was 75.02 ± 2.68%.

Discussion

PON1 is a multi-functional protein with a variety of activities such as arylesterase (AREase; hydrolysis of aromatic esters such as phenyl acetate), paraoxonase (POXase; paraoxon hydrolysis), diazoxonase (DZOase; hydrolysis of diazoxon), and lactonase activity (hydrolysis of lactones) [11, 16, 23]. Decreased PON1 activities may progress in CAD and be influenced in AF, due to its role as an antioxidant and anti-inflammatory that protects oxidative modification of HDLs and LDL in the human system [24]. These beneficial impacts are attributed to PON1's peroxidase and esterase activity, which allows the detoxification of oxidative biomolecules including phospholipids and lipid hydroperoxides [25]. Hence, the increased PON1 activity benefits CAD and AF management, which regulate oxidative stress and inflammation, which are the major contributors to atherosclerosis [26]. Recently, Argan et al. suggest that a few CAD drugs, such as lidocaine-HCl, lacidipine, propafenone, and propranolol, can highly inhibit PON1 activity [16]. On the other hand, the DOACs are a group of drugs that selectively block certain

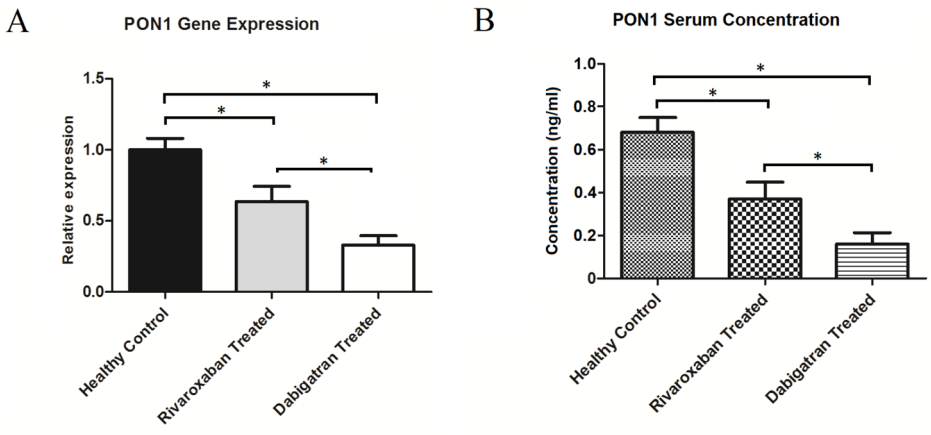

Fig. 5 Relative expression of PON1. **A**) Relative quantification of *PON1* gene expression in healthy control, rivaroxaban, and dabigatran treated participants. **B**) Influences of PON1 enzyme activity in normal healthy control, rivaroxaban, and dabigatran treated AF participants

pathways (Thrombin and Factor Xa) involved in blood clotting in order to prevent the formation of blood clots in patients with atrial fibrillation [27]. DOACs, in contrast to warfarin, exhibit more consistent pharmacokinetics, do not necessitate regular monitoring, and have fewer limitations on diet and interactions with other medications. The risk factors of DOACs associated with atrial fibrillation (AF) include bleeding, renal impairment, dyspepsia, and gastritis [28, 29]. Therefore, selecting the suitable medication for the management of AF is vital, as it is contingent upon the patient's state and medical background. Furthermore, the DOACs have the potential to interact with other medications, which may lead to an increased risk of adverse effects or a decrease in their effectiveness [30]. PON1 has an anti-inflammatory effect that involves reducing the adhesion of endothelin and the chemotaxis of monocytes [31, 32]. It also prevents the transformation of macrophages from monocytes, resulting in a decrease in the vascular inflammatory response [33]. Nevertheless, the production of PON1 occurs in the liver, and its functionality can be affected by the liver's performance [12]. Certain DOACs have the potential to induce liver function abnormalities [34], which may have an effect on the synthesis and activity of PON1. Thus, the main objective of the study is to identify the candidate DOAC for the treatment of AF that does not adversely affect the beneficial protein PON1. Henceforth, we implement series of computational (protein modeling, molecular docking, and dynamics simulation) and molecular approaches (gene expression and enzyme concentration) to investigate the impact of DOACs on the PON1 protein and to recommend better DOAC in the treatment of AF.

In our computational assessment, the PON1 structure was generated and found suitable by validation through ERRAT, VERIFY3D, and PROCHECK tools. Then, molecular docking was performed and the impact of DOACs on PON1 was determined. Molecular docking identifies best binding pose of ligand towards the protein and provide the binding energy for the ligand for the corresponding protein [35]. Least the binding energy suggest high the binding affinity, which likely to have high inhibitory effect to the target protein [35]. In this study, dabigatran showed least binding energy score, which presents a high potential inhibitory effect to PON1 protein. On the other hand, rivaroxaban demonstrated the less inhibitory effect when compared to other known DOACs. Notably, rivaroxaban interacts with the oxygen and hydroxide groups that form a two hydrogen bond with the SER193 residue contributing less binding affinity with PON1 protein. Relatively, apixaban formed a strong pi-pi interaction with the PON1 residue (PHE292). Whereas, dabigatran forms four hydrogen bonds, including GLU53, SER193, TYR197, and ASP269; salt bridge at ASP269; and it has three pi-pi stackings with TYR71, PHE222, and PHE292. Such multiple interactions between dabigatran and PON1 may have a negative impact on AF treatment efficiency. Henceforth, dabigatran has showed high inhibitory effect towards PON1 protein than apixaban and rivaroxaban. Further, MD simulation was performed until 100 ns to determine the structural stability change of PON1 protein on binding of rivaroxaban (lowest binding score) and dabigatran (highest binding score).

The evaluation of MD simulation provides protein structural conformation that is linked to protein function. In particular, ligand interaction with the protein target results in structural deviations and conformational alterations and may affect the stability of the protein [36]. MD simulation was used to analyse the interaction of protein and ligand in simulated physiological environment, the stable interaction represents that the ligand is constantly bound to protein. In MD simulation, the more stable the drug towards the target, which indicates higher inhibitory effect [36, 37]. For instance, the RMSD plot showed dabigatran with PON1 complex had a higher fluctuation

between 1 and 50 ns, and after that, the complex structure was stabilized (Fig. 2A). While the rivaroxaban has a higher fluctuation between 1 and 60 ns with PON1, then minor deviation were noticed throughout the 100 ns simulation (Fig. 2A). An increased average RMSD value was observed with rivaroxaban complex compared to dabigatran, indicating that rivaroxaban has less stable conformational towards the PON1 protein. This suggests that dabigatran achieves a more stable conformation upon binding to PON1, reflecting a more effective interaction between dabigatran and PON1. Whereas, the rivaroxaban binding contributed to protein flexibility, which conferred less binding of rivaroxaban in accordance with docking results. RMSF showed a higher deviation in the rivaroxaban and reached 0.52 nm at the position of the 76th residue, while the apo-PON1 residues showed lower fluctuations (0.20 nm). In contrast, dabigatran reaches a peak of 0.46 nm at the 186th residue (Fig. 2B) compared to the apo-PON1 (0.20 nm). The PON1 structure contains two calcium (Ca) binding sites (in the catalytic site) conserved in the PON1 sequence (Ca1: D54 and D169; Ca2: N224 and D269) [38]. Almost most Ca binding sites have a lower RMSF value, i.e., less than 1.5 nm, which indicates that the Ca binding residues remained stable during the course of the simulation without major flexibility change. Similarly, the RMSF results showed that PON1 with rivaroxaban contributed to more fluctuation at their active sites than dabigatran. Fluctuation in protein prevents the binding of rivaroxaban. In addition, the SASA demonstrates that the rivaroxaban and dabigatran with PON1 complexes have higher area than the apo-PON1 (Fig. 3B). Notably, the increase in surface area of complex structures with solvents might be due to the binding of drug. In terms of number of HB formation, the apo-PON1 and rivaroxaban-PON1 complex have 238 contacts, but dabigatran-PON1 complex showed decreased contacts (230) during 100 ns simulation. In addition, the parallel simulations were showed that rivaroxaban with the PON1 complex has lower stability than the other structures (Supplementary File 1). Overall, our computational assessment provides a significant clue that dabigatran has a greater inhibitory effect on PON1 than that of rivaroxaban. Henceforth, molecular investigation is essential to substantiate the computational outcome.

In our molecular approach, the AF participants under treatment (dabigatran and rivaroxaban) were recruited and compared with healthy controls. Our initial assessment with clinical data suggests the participants under rivaroxaban treatment had decreased LDL and other cholesterol levels compared to dabigatran (Table 1). The gene expression analysis in PBMC revealed critical insights into the regulation of *PON1* in AF patients. Our study demonstrated a significant down-regulation of *PON1* gene expression in patients with AF compared to healthy controls. This suggests that AF is associated with reduced *PON1* levels, which may contribute to increased oxidative stress and inflammation observed in the DOACs treated patients. Among AF patients, those treated with dabigatran exhibited significantly lower *PON1* expression levels compared to those treated with rivaroxaban (Fig. 5A). This indicates that dabigatran may have a more pronounced inhibitory effect on *PON1* gene expression than rivaroxaban. Similarly, the serum PON1 protein was significantly reduced in dabigatran treated AF group compared to the healthy group (Fig. 5B). The differential impact of dabigatran and rivaroxaban on PON1 (gene expression and protein level) highlights a potential advantage of rivaroxaban in preserving PON1 levels in AF patients. This observation was in accordance with our computational results suggesting the high inhibitory effect of PON1 via dabigatran.

Overall, this analysis suggests that rivaroxaban will aid in effective treatment for AF. However, the limitation of this study is that *PON1* gene expression and serum PON1 level were evaluated in a moderate sample size, so further sampling is required. Additionally, a crucial, in-depth study is needed to identify the change in molecular pathways and other essential proteins influenced by DOACs. Our results will act as a fundamental molecular study that helps to discover a candidate drug that helps in AF treatment as well as a foundation for future studies.

Conclusions

Through our computational approach, the PON1 protein structure was successfully modeled and validated to have good quality. The modeled structure was docked with the DOACs, and dabigatran had the highest binding affinity with the PON1 protein when compared to rivaroxaban. Additionally, MD simulation results confirm dabigatran has the ability to cause more structural stability in PON1 than rivaroxaban. Further experimental validation confirmed that dabigatran-treated participants had a decrease in *PON1* gene expression and reduced PON1 enzyme levels when compared to rivaroxaban. Thereby, our study suggests that rivaroxaban has the minimal effect on the PON1 compared to dabigatran through both computational and experimental investigations. Henceforth, rivaroxaban could be a suitable DOAC for the treatment of AF and to sustain the beneficial effect of PON1.

Abbreviations

CAD	Coronary artery disease
AF	Atrial fibrillation
DOACs	Direct oral anticoagulants
PON1	Paraoxonase 1
MD	Molecular dynamics
PBMC	Peripheral blood mononuclear cells
LDL	Low-density lipoproteins
HDL	High density lipoproteins

SDF	Structure data file
PME	Particle mesh Ewald
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation
SASA	Solvent accessible surface area
Rg	Radius of gyration
HB	Hydrogen bond
GROMACS	GROningen Machine for Chemical Simulation
BMI	Body mass index
TC	Total cholesterol
TG	Triglyceride
HbA1c	HaemoglobinA1c ()
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40360-024-00785-z.

Supplementary Material 1

Acknowledgements
All authors thank their institutes for the infrastructure support for this study. The authors acknowledge and extend their appreciation to the Researchers Supporting Project Number (RSPD2024R709), King Saud University, Riyadh, Saudi Arabia for funding this study.

Author contributions
SM—Investigation, Formal analysis and draft writing of the manuscript. JV, DD, and RS—Formal analysis, resource, review and editing manuscript. SFA, and SMA—Resources and review and editing manuscript. SSJ—Designed the study, Supervision, Investigation, review and editing manuscript.

Funding
This research was funded by the King Saud University (Project Number - RSPD2024R709), Riyadh, Saudi Arabia.

Data availability
Derived data supporting the findings of this study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate
This study was approved by Human Institutional Ethical committee (IHEC-II/0118/21) of Chettinad Academy of Research and Education, Chettinad Hospital and Research Institute, Kelambakkam, Tamil Nadu– 603103, India. We confirm that all methods were performed in accordance with the accordance with the Declaration of Helsinki. All participants gave written informed consent before recruitment.

Consent for publication
All authors on the research paper have approved the manuscript for submission.

Competing interests
The authors declare no competing interests.

Author details
[1]Drug Discovery and Multi-omics Laboratory, Faculty of Allied Health Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu 603103, India
[2]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[3]Heal Your Heart EECP Centers, Vaso-Meditech Private Limited, Chennai, Tamil Nadu 600041, India
[4]Department of Cardiology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu 603103, India

Received: 2 May 2024 / Accepted: 19 August 2024
Published online: 22 August 2024

References

1. Boerschel CS, Schnabel RB. The imminent epidemic of atrial fibrillation and its concomitant diseases–myocardial infarction and heart failure-a cause for concern. Int J Cardiol. 2019;287:162–73. https://doi.org/10.1016/j.ijcard.2018.11.123.
2. Wijesurendra RS, Casadei B. Mechanisms of atrial fibrillation. Heart. 2019;105(24):1860–7. https://doi.org/10.3389/fgene.2022.966413.
3. Bordignon S, Chiara Corti M, Bilato C. Atrial fibrillation associated with heart failure, stroke, and mortality. J Atr Fibrillation. 2012;5(1):467. https://doi.org/10.4022/jafib.467.
4. Huang HD, Darbar D. Genetic risk scores for atrial fibrillation: do they improve risk estimation? Eur Heart J. 2017;33(4):422–4. https://doi.org/10.1016/j.cjca.2016.12.006.
5. Kloosterman M, Santema BT, Roselli C, Nelson CP, Koekemoer A, Romaine SP, Rienstra M. Genetic risk and atrial fibrillation in patients with heart failure. Eur J Heart Fail. 2020;22(3):519–27. https://doi.org/10.1002/ejhf.1735.
6. Bosch NA, Cimini J, Walkey AJ. Atrial fibrillation in the ICU. Chest. 2018;154(6):1424–34. https://doi.org/10.1016/j.chest.2018.03.040.
7. Bortman LV, Mitchell F, Naveiro S, Pérez Morales J, Gonzalez CD, Di Girolamo G, Giorgi MA. Direct oral anticoagulants: an updated systematic review of their clinical pharmacology and clinical effectiveness and safety in patients with nonvalvular atrial fibrillation. J Clin Pharmacol. 2023;63(4):383–96. https://doi.org/10.1002/jcph.2184.
8. Undas A, Drabik L, Potpara T. Bleeding in anticoagulated patients with atrial fibrillation: practical considerations. Kardiol Pol. 2020;78(2):105–16. https://doi.org/10.33963/KP.15205.
9. Chan YH, See LC, Tu HT, Yeh YH, Chang SH, Wu LS, et al. Efficacy and safety of apixaban, dabigatran, rivaroxaban, and warfarin in Asians with nonvalvular atrial fibrillation. J Am Heart Assoc. 2018;7(8):e008150. https://doi.org/10.1161/JAHA.117.008150.
10. Mekaj YH, Mekaj AY, Duci SB, Miftari EI. New oral anticoagulants: their advantages and disadvantages compared with vitamin K antagonists in the prevention and treatment of patients with thromboembolic events. Ther Clin Risk Manag. 2015;11:967–77. https://doi.org/10.2147/TCRM.S84210.
11. Vavlukis M, Vavlukis A, Krsteva K, Topuzovska S. Paraoxonase 1 gene polymorphisms in lipid oxidation and atherosclerosis development. Front Genet. 2022;13:966413.
12. Shokri Y, Variji A, Nosrati M, Khonakdar-Tarsi A, Kianmehr A, Kashi Z, et al. Importance of paraoxonase 1 (PON1) as an antioxidant and antiatherogenic enzyme in the cardiovascular complications of type 2 diabetes: genotypic and phenotypic evaluation. Diabetes Res Clin Pract. 2020;161:108067. https://doi.org/10.1016/j.diabres.2020.108067.
13. Ghoreshi ZA, Abbasi-Jorjandi M, Asadikaram G, Sharif-Zak M, Seyedi F, Khaksari Haddad M, et al. Paraoxonase 1 rs662 polymorphism, its related variables, and COVID-19 intensity: considering gender and post-COVID complications. Exp Biol Med (Maywood). 2023;248(23):2351–62. https://doi.org/10.1177/15353702221128563.
14. Litvinov D, Mahini H, Garelnabi M. Antioxidant and anti-inflammatory role of paraoxonase 1: implication in arteriosclerosis diseases. N Am J Med Sci. 2012;4(11):523–32. https://doi.org/10.4103/1947-2714.103310.
15. Mackness M, Mackness B. Human paraoxonase-1 (PON1): gene structure and expression, promiscuous activities and multiple physiological roles. Gene. 2015;567(1):12–21. https://doi.org/10.1016/j.gene.2015.04.088.
16. Argan O, Cikrikci K, Uslu H, Gencer N. In vitro effects of thirty-eight cardiac drugs on human serum paraoxonase. Chem Biol Drug Des. 2022;100(1):80–9. https://doi.org/10.1111/cbdd.14054.
17. Xiang Z. Advances in homology protein structure modeling. Curr Protein Pept Sci. 2006;7(3):217–27. https://doi.org/10.2174/138920306777452312.
18. Bryer AJ, Reddy T, Lyman E, Perilla JR. Full scale structural, mechanical, and dynamical properties of HIV-1 liposomes. PLoS Comput Biol. 2022;18(1):e1009781. https://doi.org/10.1371/journal.pcbi.1009781.

19. Boonstra S, Onck PR, Giessen EV. CHARMM TIP3P water model suppresses peptide folding by solvating the unfolded state. J Phys Chem B. 2016;120(15):3692–8. https://doi.org/10.1021/acs.jpcb.6b01316.
20. Kirubhanand C, Merciline Leonora J, Anitha S, Sangeetha R, Nachammai KT, Langeswaran K, et al. Targeting potential receptor molecules in non-small cell lung cancer (NSCLC) using in-silico approaches. Front Mol Biosci. 2023;10:1124563. https://doi.org/10.3389/fmolb.2023.1124563.
21. Parrinello M, Rahman A. Polymorphic transitions in single crystals: a new molecular dynamics method. J Appl Phys. 1981;52:7182–90. https://doi.org/10.1063/1.328693.
22. Hess B, Bekker H, Berendsen HJ, Fraaije JG. LINCS: a linear constraint solver for molecular simulations. J Comput Chem. 1997;18:1463–72. https://doi.org/10.1021/ct700200b.
23. Richter RJ, Jarvik GP, Furlong CE. Paraoxonase 1 (PON1) status and substrate hydrolysis. Toxicol Appl Pharmacol. 2009;235(1):1–9. https://doi.org/10.1016/j.taap.2008.11.001.
24. Varadhan S, Venkatachalam R, Perumal SM, Ayyamkulamkara SS. Evaluation of oxidative stress parameters and antioxidant status in coronary artery disease patients. Arch Razi Inst. 2022;77(2):853–8. https://doi.org/10.22092/ARI.2022.357069.1965.
25. Asare GA, Andam SE, Asare-Anane H, Ammanquah S, Anang-Quartey Y, Afriyie DK, Musah I. Lipid-associated antioxidants: arylesterase and paraoxonase-1 in benign prostatic hyperplasia treatment-naïve patients. Prostate Int. 2018;6(1):36–40. https://doi.org/10.1016/j.prnil.2017.04.002.
26. Shekhanawar M, Shekhanawar SM, Krisnaswamy D, Indumati V, Satishkumar D, Vijay V, et al. The role of 'paraoxonase-1 activity' as an antioxidant in coronary artery diseases. J Clin Diagn Res. 2013;7(7):1284. https://doi.org/10.7860/JCDR/2013/5144.3118.
27. Kustos SA, Fasinu PS. Direct-acting oral anticoagulants and their reversal Agents-An update. Medicines (Basel). 2019;6(4):103. https://doi.org/10.3390/medicines6040103.
28. Rogula S, Gąsecka A, Mazurek T, Navarese EP, Szarpak Ł, Filipiak KJ. Safety and efficacy of DOACs in patients with advanced and end-stage renal disease. Int J Environ Res Public Health. 2022;19(3):1436. https://doi.org/10.3390/ijerph19031436.
29. Issever K, Kuloglu E, Muhtaroglu A, Seker D, Kotur O, Dulger AC. The effect of direct oral anticoagulants on gastric mucosa and Helicobacter Pylori prevalence in dyspeptic patients: a retrospective cross-sectional study. Cureus. 2023;15(10). https://doi.org/10.7759/cureus.46477.
30. Capiau A, Mehuys E, De Bolle L, Van Tongelen I, De Backer T, Boussery K. Drug-drug interactions with direct oral anticoagulants: development of a consensus list for ambulatory care. Int J Clin Pharm. 2023;45(2):364–74. https://doi.org/10.1007/s11096-022-01511-7.
31. Durrington PN, Bashir B, Soran H. Paraoxonase 1 and atherosclerosis. Front Cardiovasc Med. 2023;10:1065967. https://doi.org/10.3389/fcvm.2023.1065967.
32. Sirca TB, Mureșan ME, Pallag A, Marian E, Jurca T, Vicaș LG, et al. The role of polyphenols in modulating PON1 activity regarding endothelial dysfunction and atherosclerosis. Int J Mol Sci. 2024;25(5):2962. https://doi.org/10.3390/ijms25052962.
33. Aharoni S, Aviram M, Fuhrman B. Paraoxonase 1 (PON1) reduces macrophage inflammatory responses. Atherosclerosis. 2013;228(2):353–61. https://doi.org/10.1016/j.atherosclerosis.2013.03.005.
34. Juneja D, Nasa P, Jain R. Liver injury from direct oral anticoagulants. World J Hepatol. 2023;15(6):841–9. https://doi.org/10.4254/wjh.v15.i6.841.
35. Jauslin WT, Schild M, Schaefer T, Borsari C, Orbegozo C, Bissegger L, et al. A high affinity pan-PI3K binding module supports selective targeted protein degradation of PI3Kα. Chem Sci. 2023;15(2):683–91. https://doi.org/10.1039/d3sc04629j.
36. Pandi S, Kulanthaivel L, Subbaraj GK, Rajaram S, Subramanian S. Screening of potential breast cancer inhibitors through molecular docking and molecular dynamics simulation. Biomed Res Int. 2022;3338. https://doi.org/10.1155/2022/3338549.
37. Sinha S, Tam B, Wang SM. Applications of molecular dynamics simulation in protein study. Membranes (Basel). 2022;12(9):844. https://doi.org/10.3390/membranes12090844.
38. Ahmad MSVJ, Attia SF, Emran SM, Patil TB, Ahmed RB. Structural characteristics of PON1 with Leu55Met and Gln192Arg variants influencing oxidative-stress-related diseases: an integrated molecular modeling and dynamics study. Medicina (Kaunas). 2023;59(12):2060. https://doi.org/10.3390/medicina59122060.

Publisher's note