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Molecular Docking Analysis of the Affinities of Lipid-Lowering Drugs to Paraoxonase-1 Enzyme and Its Polymorphic Structures

Lipid Düşürücü İlaçların Paraoksonaz-1 Enzimine ve Polimorfik Yapılarına Afinitelerinin Moleküler Docking Analizi

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ABSTRACT

Paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-associated enzyme that exhibits paraoxonase, arylesterase, and lactonase activities. This multifunctional enzyme plays a crucial role in preventing atherosclerosis by inhibiting low-density lipoprotein (LDL) oxidation and reducing oxidized lipid levels. The present study aimed to investigate the affinities of various lipid-lowering drugs to PON1 and its polymorphic structures [(M/L)55 and (Q/R)192] using advanced molecular docking methods. The research utilized a comprehensive computational approach, including homology modeling, molecular dynamics simulation, and AutoDock 4 software to analyze the interactions between PON1 and several classes of lipid-lowering agents. These included statins (simvastatin, atorvastatin, lovastatin, mevastatin, fluvastatin, rosuvastatin, pravastatin), fibrates (fenofibrate, gemfibrozil, bezafibrate, ciprofibrate), niacin, ezetimibe, orlistat, sibutramine, probucol, and phytosterols (brassicasterol, campesterol, β-sitosterol, stigmasterol). The study revealed varying affinities of these drugs to PON1 and its polymorphic structures. Notably, brassicasterol showed the highest affinity for the normal PON1 structure, while sibutramine and stigmasterol demonstrated the highest affinities for the Q/R 192 and M/L 55 polymorphic structures, respectively. Conversely, orlistat exhibited the lowest affinity for both normal PON1 and the M/L 55 polymorphic structure, while atorvastatin showed the lowest affinity for the Q/R 192 polymorphic structure. These findings provide valuable insights into the potential interactions between lipid-lowering drugs and PON1, suggesting that consideration of PON1 affinity might be important in the selection of lipid-lowering therapies, particularly in individuals with different PON1 polymorphisms. However, further *in vitro* and *in vivo* studies are necessary to validate these computational results and establish their clinical relevance.

Keywords: Cardiovascular disease, Lipid-lowering drugs, Molecular docking, Paraoxonase-1 (PON1), PON1 polymorphisms

ÖZET

Paraoksonaz-1 (PON1), paraoksonaz, arilesteraz ve laktonaz aktiviteleri gösteren, yüksek yoğunluklu lipoprotein (HDL) ile ilişkili bir enzimdir. Bu çok fonksiyonlu enzim, düşük yoğunluklu lipoprotein (LDL) oksidasyonunu önleyerek ve oksitlenmiş lipid seviyelerini azaltarak aterosklerozun önlenmesinde önemli bir rol oynamaktadır. Bu çalışma, çeşitli lipid düşürücü ilaçların PON1 ve polimorfik yapılarına [(M/L)55 ve (Q/R)192] olan afinitelerini gelişmiş moleküler doking yöntemleri kullanarak araştırmayı amaçlamıştır. Araştırma, PON1 ile çeşitli lipid düşürücü ajanlar arasındaki etkileşimleri analiz etmek için homoloji modellemesi, moleküler dinamik simülasyonu ve AutoDock 4 yazılımını içeren kapsamlı bir hesaplamalı yaklaşım kullanmıştır. Bu ajanlar arasında statinler (simvastatin, atorvastatin, lovastatin, mevastatin, fluvastatin, rosuvastatin, pravastatin), fibratlar (fenofibrat, gemfibrozil, bezafibrat, siprofibrat), niasin, ezetimib, orlistat, sibutramin, probukol ve fitosteroller (brasikasterol, kampesterol, β-sitosterol, stigmasterol) yeralmaktadır. Çalışma, builaçların PON1 ve polimorfik yapılarına değişen afiniteler gösterdiğini ortaya koymuştur. Özellikle, brasikasterol normal PON1 yapısına en yüksek afiniteyi gösterirken, sibutramin ve stigmasterol sırasıyla Q/R 192 ve M/L 55 polimorfik yapılarına en yüksek afiniteleri göstermiştir. Buna karşılık, orlistat hem normal PON1 hem de M/L 55 polimorfik yapısına en düşük afiniteyi gösterirken, atorvastatin Q/R 192 polimorfik yapısına en düşük afiniteyi göstermiştir. Bu bulgular, lipid düşürücü ilaçlar ile PON1 arasındaki potansiyel etkileşimler hakkında değerli bilgiler sağlamakta ve PON1 afinitesinin, özellikle farklı PON1 polimorfizmleri olan bireylerde lipid düşürücü tedavilerin seçiminde önemli olabileceğini göstermektedir. Bununla birlikte, bu hesaplamalı sonuçları doğrulamak ve klinik önemini belirlemek için daha fazla *in vitro* ve in vivo çalışma gereklidir.

Anahtar Kelimeler: Kardiyovasküler hastalık, Lipid düşürücü ilaçlar, Moleküler doking, Paraoksonaz-1 (PON1), PON1 polimorfizmleri

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INTRODUCTION

Paraoxonase-1 (PON1) has emerged as a subject of intense research interest in recent years, primarily due to its pivotal role in lipid metabolism and cardiovascular health. As an enzyme associated with high-density lipoprotein (HDL), PON1 exhibits a remarkable ability to hydrolyze a wide range of substrates, including oxidized lipids, homocysteine thiolactone, and various toxic organophosphate compounds.[1,2] The enzyme's capacity to prevent low-density lipoprotein (LDL) oxidation and reduce oxidized lipid levels has positioned it as a key player in the prevention of atherosclerosis and, by extension, cardiovascular diseases.[1]

The PON1 gene, located on chromosome 7 in humans, is known to have several polymorphisms that can affect the enzyme's activity and concentration in the serum. Two of the most studied polymorphisms are the (M/L)55 and (Q/R)192 variants, which have been associated with varying levels of enzymatic activity and different susceptibilities to cardiovascular diseases.[3] These genetic variations add a layer of complexity to the study of PON1 and its interactions with various compounds, including lipid-lowering drugs.

Lipid-lowering drugs represent a cornerstone in the management of dyslipidemia and the prevention of cardiovascular diseases. This diverse group of pharmaceuticals includes several classes of compounds, each with unique mechanisms of action.[4] While the primary mechanisms of these drugs in lipid lowering are well established, their potential interactions with other physiological systems, including enzymes like PON1, are not fully understood. The relationship between lipid-lowering drugs and PON1 has been a subject of investigation, with some studies reporting conflicting results regarding the effects of these drugs on PON1 activity.[5,6]

The advent of computational methods in drug discovery and molecular biology has opened new avenues for investigating such complex interactions. Molecular docking, in particular, has emerged as a powerful tool for predicting the binding affinities and orientations of small molecules to their target proteins. This in silico approach allows for the rapid screening of multiple compounds and can provide valuable insights into potential drug-enzyme interactions, guiding further experimental studies and potentially informing clinical decision-making.[7]

The present study aims to leverage these computational techniques to examine the affinities of various lipid-lowering drugs to PON1 and its polymorphic forms by utilizing molecular docking methods. This comprehensive in silico analysis aims to contribute to our understanding of the complex interplay between lipid-lowering drugs and PON1, potentially shedding light on the broader implications of these interactions in the context of cardiovascular health and personalized medicine.

METHODS

Ligands and paraoxonase protein

Lipid-lowering drugs such as ezetimibe, ciprofibrate, clofibrate, fenofibrate, gemfibrozil, beta-sitosterol, brassicasterol, campesterol, stigmasterol, bezafibrate, niacin, orlistat, probucol, sibutramine, atorvastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin have been used. All ligands had hydrogen atoms added using Marvin Sketch software before processing (Marvin 5.6.0.5, 2011, (ChemAxon). Each ligand underwent a total of 100,000 steps of minimization using the mmff94 (Merck Molecular Force Field) force field and the "steepest descent" optimization algorithm.[8] Ligands were charged using the ADT (Autodock Tools) program with Gasteiger, and all bonds except amide bonds were set to be freely adjustable. All ligands were obtained from the PubChem ligand database.[9,10] The modeling, simulation, and docking methodology of Paraoxonase Protein was established based on the previous work conducted by Duzgun et al.[11]

Hardware

All docking and molecular dynamics simulations were conducted on TÜBİTAK's high-performance computing clusters, Trgrid TRUBA. Each of the computer clusters used in Trgrid consists of nodes with two 12-core "Opteron 6174" processors, making a total of 24 cores. Molecular dynamics simulations were run in parallel across multiple clusters using GROMACS 4.5.5 software.[12] Docking processes were performed on a computer with a 4-core Intel Core i3 processor using "Autodock 4" and "Autodock Vina" software.[13,14]

Modeling of paraoxonase protein

The protein structure modeling process began with the PON1 protein structure (PDB code: 3SRE) obtained from the Protein Data Bank.[15] The structure was preprocessed using Chimera software to remove existing ligands, preparing it for subsequent homology modeling.[16] Homology modeling was performed using

MODELLER software, with the human serum paraoxonase enzyme sequence from Uniprot (accession code: P27169) serving as the template.[17,18] The sequence alignment showed 85% identity between the template and target sequences, indicating a high probability of accurate model generation. To study the polymorphic variants, specific amino acid modifications were made at positions 55 and 192 to create the Q/R192 and M/L55 variants. For each variant (wild-type, Q/R192, and M/L55), MODELLER generated 20 distinct models. The modeling process included the retention of Ca^{2+} cofactors by enabling the 'include HETATM residues other than water' option. The calcium ions were maintained in their crystallographic positions due to their critical role in structural stability and catalytic function. The final model selection for each variant was based on quality assessment using the Molprobity server, with the highest-scoring model chosen for further analysis.[19]

Molecular dynamics simulation

All molecular dynamics calculations were conducted on the computer clusters on Trgrid, each with 24 cores (AMD Opteron) using GROMACS 4.5.5 software.[20] The parallel computing setup allowed for efficient handling of the computationally intensive simulations. The structures obtained in PDB file format from the previously used "MODELLER" homology modeling software were subjected to a series of stages according to the following diagram. The simulation box was constructed with periodic boundary conditions using a dodecahedron geometry, with a minimum distance of 1.2 nm between the protein and box edges. The "forcefield" used was AMBER99SB-ILDN, and SPC (simple point charge) was chosen as the water model.[21,22] Na^+ and Cl^- ions were added for neutralization of the system. The ionic strength was adjusted to 0.15 M to mimic physiological conditions. A total of 5000 steps of the "steepest descent" minimization algorithm were performed. The minimization was continued until the maximum force was less than 1000 kJ/mol/nm. The equilibrium phase occurred in two phases, NVT (for temperature and volume stability) and NPT (for pressure and density stability). Initially, a 100 ps NVT phase was initiated. The other phase was a two-step NPT phase. The first step was a 100 ps phase using position restraining algorithms, while the second step was a 1 ns final equilibrium phase without position restraining algorithms. A 10 ns simulation was applied in the production phase. To ensure that the simulation was successfully conducted, several data analyses were performed. One of these was structural stability, which was evaluated using RMSD calculations. RMSF analysis was conducted to show the mobility of each residue in the protein. The radius of gyration (Rg) is a measure of the compactness of a protein; if a protein is stably folded, it will show a certain stable Rg value in the corresponding graph.

Molecular docking

Docking was performed using two different computational algorithms: Autodock4 and Autodock Vina.[13,14] Both programs were chosen for their complementary strengths in binding prediction and scoring functions. The human PON1 model, obtained from the rabbit PON1 enzyme through homology modeling, underwent MD simulation with GROMACS software to gain appropriate structure and behavior under in vivo conditions. The final structure for docking was selected from the MD trajectory based on clustering analysis of conformations. The flexible missing residues in the range of 72-81 in the 3SRE model were added in this process. The Y71 and R292 residues were treated as flexible in both docking processes. The docking procedure used for AutoDock 4.2 was based on the protocol established by Ben-David et al. In AutoDock 4.2, the active site of the PON1 enzyme was targeted with an average grid volume of 39 Å (Angstrom) for the docking process. Grid maps were generated with 0.375 Å spacing. In AutoDock Vina, the docking process was similarly based on the active site of the PON1 enzyme, with an average grid volume of 17 Å (Angstrom). Exhaustiveness was set to 4. Vina and AutoDock 4.2 considered all ligands as flexible except for the Y71 and R292 residues. The electrostatic field was calculated on a 1 Å grid, and all other settings related to Vina were left as default. The docking results were analyzed based on binding energy scores and clustering of binding poses. AutoDock 4 was preferred due to its ability to calculate the Ca^{2+} cofactor and electrostatic charge of the protein. Since AutoDock Vina could not perform these calculations, resulting in poor correlation and incorrect conformations, only AutoDock 4 was used for affinity calculations of the drugs. The final binding poses were selected based on both energy scores and visual inspection of the protein-ligand interactions.

RESULTS

Molecular dynamics simulation

A molecular dynamics study was conducted using Gromacs software on PON1 protein and its polymorphic

structures. The protein systems, with an average molecular weight of 39.75 kDa, each contained two calcium atoms as cofactors. The analysis focused primarily on RMSD, RMSF and Radius of gyration (Rg) measurements. RMSD, measured using alpha carbon positions, serves as an indicator of system stability and structural integrity. A stable RMSD value suggests system equilibration. The MD simulations revealed distinct conformational patterns. All systems showed an initial RMSD increase from 0.05 nm during the first 2 ns. The wild-type system stabilized between 0.15-0.17 nm after 6 ns. The M/L 55 variant showed lower RMSD values (0.13-0.15 nm), indicating increased structural rigidity. The Q/R 192 variant displayed the highest RMSD values (0.16-0.19 nm) with greater fluctuations, suggesting enhanced flexibility. All systems reached equilibrium within 6-7 ns, with M/L 55 showing the most stable trajectory. Protein flexibility was analyzed using RMSF calculations, which measure residue-specific mobility throughout the simulation. The analysis identified key structural regions: loops (L1, L2, L3) and helices (H1, H2). The N-terminal H1 region showed maximum flexibility, followed by the L1 region near the active site. Rg measurements provided insights into protein compactness. The wild-type maintained an Rg around 1.91 nm, while both variants showed slightly higher values around 1.93 nm. The M/L 55 and Q/R 192 variants demonstrated similar Rg values but with different fluctuation patterns. All systems maintained stable Rg values within ±0.01 nm after equilibration, indicating that while mutations caused subtle structural changes, they did not induce major conformational alterations. The higher Rg values in mutant systems suggest slightly less compact structures compared to the wild type, potentially affecting their functional dynamics.

Molecular docking

After 10 ns of molecular dynamics simulation, docking was performed using Autodock 4.2 on a total of 22 lipid-lowering drugs for each polymorphic structure of the proteins at 0.1 ns intervals starting from the 9th ns. As seen in figure 1, the best interaction was determined to be brassicasterol with the Normal PON1 protein structure, sibutramine with the Q/R 192 PON1 polymorphic structure, and stigmasterol with the M/L 55 PON1 polymorphic structure. When examining the interaction of brassicasterol with the Normal PON1 structure, no hydrogen bond formation was observed, while electrostatic interactions were observed with His285 and Leu267 (Figure 2A). When examining the interaction of sibutramine with Q/R 192 PON1, only Phe77 was observed to engage in electrostatic interaction (Figure 2B). When examining the interaction of stigmasterol with M/L 55 PON1, it was observed that the hydroxyl group at the end of the compound's steroid structure formed electrostatic interactions with Asn168 and Asn224. Additionally, His115, Glu53, and Asp269 were also observed to participate in electrostatic interactions (Figure 2C).

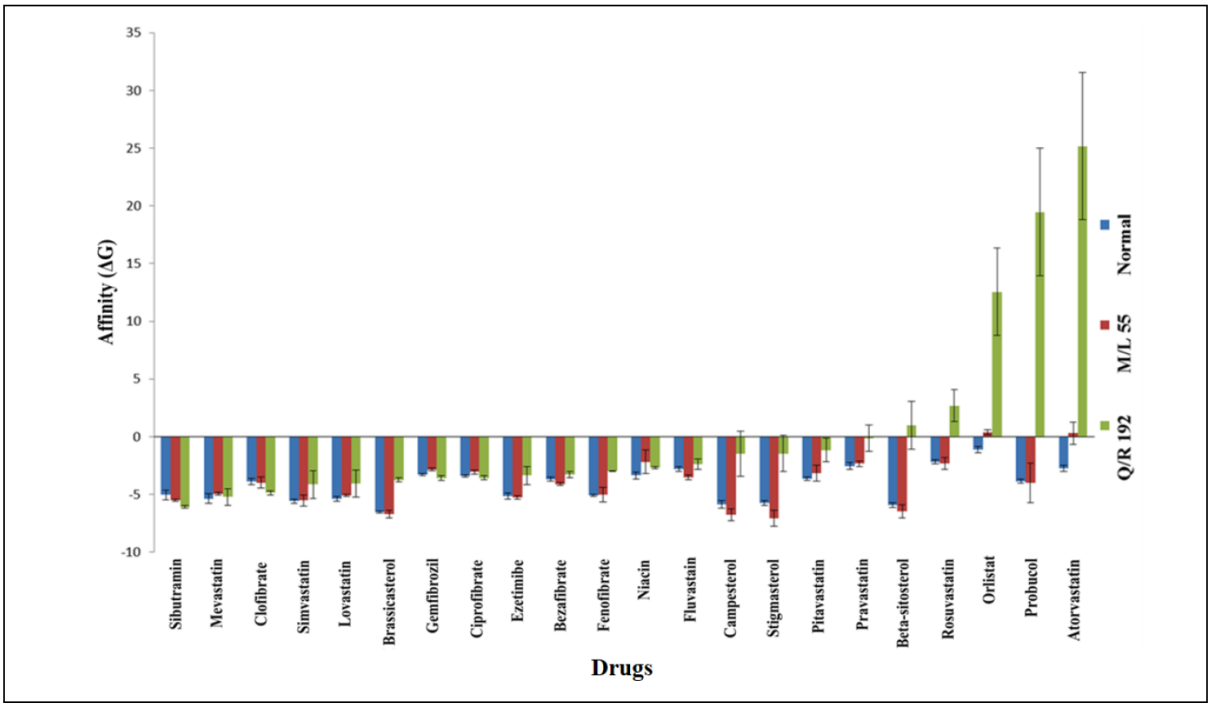

Figure 1. Affinity values of lipid-lowering drugs to PON1 and its polymorphic structures

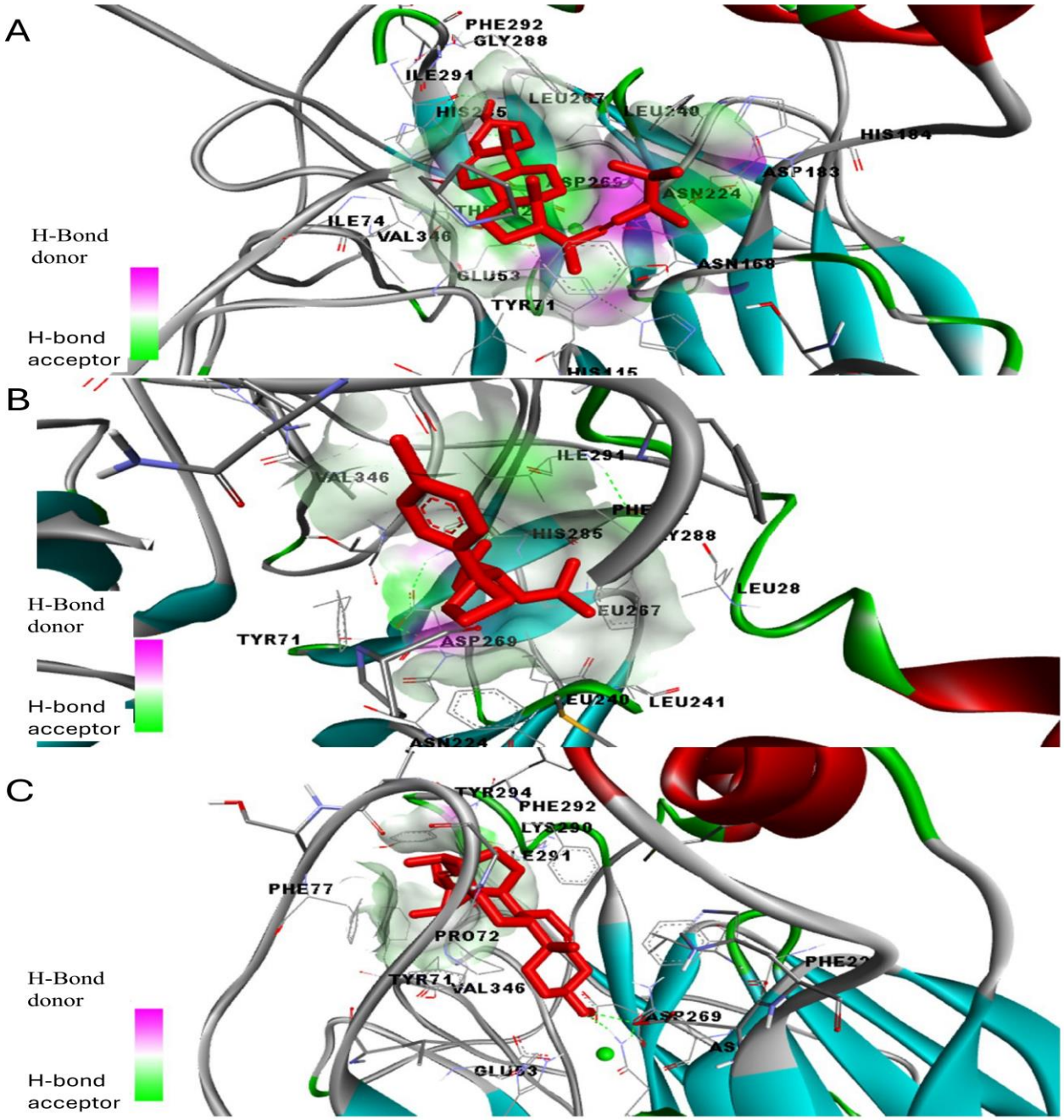

Figure 2. A- Conformation of brassicasterol shown in red structure in the active center of Normal PON1
B- Conformation of sibutramine shown in red structure in the active center of Q/R 192 PON1
C- Conformation of stigmasterol shown in red structure in the active center of M/L 55 PON1

DISCUSSION

In this study, the affinities of lipid-lowering drugs to PON1 enzyme were evaluated by applying *in silico* (computer-aided) approaches, which have been widely used in recent years to guide research before experimental studies and to save time and money. The affinities of PON1 enzyme substrates and lipid-lowering drugs were compared. Before proceeding to the molecular docking method used in affinity determination, the three-dimensional molecular structure of the PON1 enzyme was created by considering its polymorphic structures. Since the X-

RAY crystallographic structure of human PON1 enzyme was not revealed, 100% human PON1, M/L 55 and Q/R 192 polymorphic structures were obtained by homology modeling from a human-rabbit hybrid X-RAY crystal structure (PDB code: 3SRE) with 83.66% similarity at the amino acid level with the same enzymatic activity. These structures were subjected to 10 ns molecular dynamics simulation to give them their natural conformation and behavior under laboratory conditions. The characteristics of serum PON1 enzyme and (M/L) 55, (Q/R) 192 polymorphic structures were analyzed by molecular dynamics simulation.

Homology Modeling

In this study, 100% human serum PON1 protein structure and (M/L) 55, (Q/R) 192 polymorphic structures were obtained from rePON1-G2E6, a recombinant PON1 variant with identical enzymatic activity (83.66% similar to human serum paraoxonase) using homology modeling.[15] The normal and polymorphic structures generated by MODELLER, a homology modeling tool, and the experimentally obtained rePON1-G2E6 variant were aligned using the "matchmaker" tool with Chimera software. The alignment resulted in RMSD values of 0.133 with the PON1 normal construct, 0.155 RMSD with PON1 (M/L) 55 and 0.141 RMSD with PON1 (Q/R) 192. Very low RMSD values indicate that the construct produced has very high structural similarity with the experimental G2E6 variant. A value close to 0 increases the significance considerably.[23] The most deviation occurred in the L1 knot region of the PON1 enzyme close to the catalytic site. Since the knot in this region of the protein is very flexible, it could not be shown in the experimentally obtained rePON1-G2E6 variant and remained as a missing residue.[15] The MODELS tool provides a great advantage by not only producing 100% human serum PON protein and polymorphic structures, but also complementing these missing residues. MODELLER is one of the most widely used, reliable and fully automated comparative homology modeling programs. Its speed compared to other homology modeling software has made it useful for whole genome modeling studies.

In genetic, cell and molecular biology studies, experimental methods are applied to reveal protein structures. However, due to the lack of information on the atomic structure of these proteins, their molecular functions and mechanisms cannot be fully elucidated. Current methods to obtain biomolecules at atomic resolution (X-ray crystallography and NMR spectroscopy) require the preparation of high concentrations of pure proteins under physiological conditions. NMR spectroscopy can be applied for proteins with a maximum size of 15 kDa. However, many biologically important proteins have larger structures. Homology modeling can reveal the structures of proteins with different polymorphic and mutated structures.[24]

Molecular Dynamics Simulation

After homology modeling of the PON1 enzyme and its polymorphic structures were established, molecular dynamics simulations were performed. Molecular dynamics simulations were performed for 10 ns each on the normal, M/L 55 and Q/R 192 polymorphic structures of PON enzyme. With these simulations, conformational changes were examined by giving the protein its unique dynamic character in the system and molecular docking was performed with its substrates and lipid-lowering drugs on protein conformations at certain time intervals.

In this study, Parinello-Rahman' method was used to adjust the temperature, pressure and density values in molecular dynamics simulation. One of the reliability criteria of molecular dynamics simulation is the density value of the system. The density of a system containing water, ions and protein should be close to 1000 kg/m^3 in accordance with laboratory conditions.[25] In our study, the density of the system containing all three protein structures was very close to the laboratory conditions. Density differences of less than 1% between the systems were predicted to be related to the different numbers and types of atoms in the systems. In addition, small temperature and pressure differences between the systems also have an effect on the density.[25]

Over a period of 10 ns, the positional changes of each amino acid in the polymorphic structures are shown. Amino acids in the range of 70-80% are very flexible and located very close to the active site of the enzyme. This raises the question of whether the position of this region is effective in enzyme-substrate interactions. As a matter of fact, a detailed study on the enzyme PON suggested that this region may have a substrate-selective character and especially the 71st tyrosine residue may act as a cap in enzyme-substrate complexes.[26]

The choice of an appropriate energy function to describe intramolecular and intermolecular interactions is critical for a successful molecular dynamics simulation. Energy functions are usually composed of many parametric

terms. These parameters are mainly obtained from experimental and quantum mechanical studies of small molecules or fragments. Groups of functions associated with parameter settings are expressed by the term force field.[27] The force field parameters, which are vital for molecular dynamics simulations, are now being developed with the help of quantum mechanical calculations and continue to be improved with higher accuracy. AMBER (Energy Simplification Assisted Model Building) is a family of force fields for molecular dynamics simulations of biomolecules developed by the Peter Kollman group at the University of California, San Francisco. The correlation of various force fields of the AMBER family with experimental data was shown by Hornak *et al*.[28] In this study, a high correlation between the experimental NMR parameters and the parameters generated by the ff99SB force field was shown with 0.83 for lysozyme and 0.95 for ubiquitin.[28]

Molecular Docking

The most widely used Autodock program was used to determine the affinity of different polymorphic structures of PON1 with drugs and its natural substrates. While the interactions of PON1 with various substrates have been studied in depth with the docking method, there is no docking study on its affinity or interaction with lipid-lowering drugs in the literature. Xin Hu *et al*. used molecular docking, MD simulation and free energy calculation methods to investigate the interactions between the PON1 enzyme and its various substrates such as esters, lactones and phosphotrioester.[29] In their study, they showed that tyrosine 71 residue may have an important role in the binding of substrates and suggested that it may have a gate function that facilitates substrate identification. In our study, it was observed that tyrosine 71 residue has a gating function but has no direct catalytic effect in the interaction with substrates. Ben-David *et al*. and Harel *et.al*. showed that in the structure at pH 4.5 (PDB:1V04), residue 71 was close to the catalytic site, while at pH 6.5 (PDB:3SRE, 3SRG), residue 71 was outside the active site.[15,30] In other words, it has been shown that the 71st residue shows open or closed conformation at different pH. Since we used 3SRE-derived structures (operating at neutral pH) in our study, it is possible that residue 71 showed mostly open conformation.[15] A study on the structure and activity of PON1 revealed that calcium is a vital co-factor in catalytic activity.[31] As a matter of fact, in the conformations of the substrates obtained by molecular docking method on different polymorphic structures of PON1, calcium has an important effect on the formation of the enzyme-substrate complex by attracting the oxygen in the lactone structure towards itself. The Q/R 192 polymorphic structure had a narrower active center and the oxygens of its substrates were located closer to the catalytic calcium than the other polymorphic structures.

Lipid Lowering Drugs and Paraoxonase

The affinity of lipid-lowering drugs for PON1 itself and its polymorphic structures was shown in figure 1. When the comparison of the drugs between the PON structures was made, it was observed that the affinity of the drugs in the Q/R 192 polymorphism was generally lower than the other PON structures. The Y71 residue, which is located in the lid position of the active site, is open in other structures of paraoxonase, while it is closed in the Q/R 192 polymorphic structure. This may have resulted in lower affinity of the drugs. In contrast to other drugs, atorvastatin, probucol, orlistat, rosuvastatin and betasterol showed positive ΔG in the Q/R 192 polymorphic structure. In other structures of paraoxonase, atorvastatin, probucol, orlistat, rosuvastatin and pravastatin were found to have low affinity for PON1. Therefore, these drugs may not be very effective on paraoxonase activity. This suggests that drugs with high affinity may decrease paraoxonase activity, whereas drugs without any affinity or with low affinity may not affect paraoxonase activity much.

In this study, it was observed that atorvastatin could not affect paraoxonase activity, while no study was found that atorvastatin decreased or did not affect paraoxonase. In fact, it has been reported to increase PON1 activity in many studies. Kural *et al.* found that atorvastatin significantly increased serum paraoxonase activity and HDL levels in a study with dyslipidemic patients.[32,33] Similarly, Harangi *et al.* observed that atorvastatin treatment increased paraoxonase activity.[34] According to Oranje *et al.* atorvastatin decreased LDL oxidation in type 2 diabetic patients.[35] These studies pointed that atorvastatin has an important effect in preventing atherosclerotic diseases. However, Bergheanu *et al* investigated the effect of rosuvastain and atorvastatin on PON1 activity in men with cardiovascular disease and showed that both drugs increased PON1 activity, but rosuvastatin, unlike atorvastatin, increased PON1 activity in a dose-dependent manner.[36] In our study, it was observed that rosuvastatin has a weak affinity for PON1, so it may not be effective. We could not find any studies showing that

rosuvastatin reduces PON1 activity. Orlistat, another drug used in this study, showed very little affinity for PON1 in normal structure but not in polymorphic structures. Audikovszky *et al.* expressed that orlistat increased paraoxonase activity.[37]

Among the statins, simvastatin, lovastatin and mevastatin were found to have the highest affinity values. The fact that these drugs have lactone structures is the probable reason for this result. Due to this similarity, it is expected that PON activities would decrease by competitive inhibition. Consistent with our study, Billecke *et al.* reported that these three statin group drugs showed affinity for PON1 and were metabolized by PON1.[38] Another study also identified that statins such as pravastatin, fluvastatin and simvastatin reduced PON1 activity.[39] On the other hand, Tomas *et al.* stated that simvastatin increased paraoxonase activity and therefore may have actioxidant properties.[40] In a meta-analysis study conducted by Farretti *et al*., it was shown that statin therapy provides cardiovascular benefits by increasing PON1 paraoxonase and arylesterase activities, and this could be among the lipid-independent pleiotropic effects.The fact that this effect is independent of statin dose, treatment duration, or changes in LDL cholesterol levels indicates additional mechanisms underlying the cardiovascular protective effects of statins.[41] In our study, while mevastatin and simvastatin were calculated to have stronger interactions with PON1, atorvastatin was observed to be unable to interact with PON1, consistent with the study conducted by Farretti *et al.*[41]

Among all drug groups, the highest affinity was found in brassicasterol with PON1-normal structure, stigmasterol with M/L 55 polymorphic structure and sibutramine with Q/R 192 polymorphic structure. Therefore, these drugs may be effective in decreasing PON1 activity. Phytosterols showed high affinity for PON1 and M/L 55 polymorphic structure unlike fibrate type drugs. In the Q/R 192 polymorphic structure, the Y71 residue and the narrow structure of the active center together with the relatively large molecules of phytosterols may have caused them to show low activity. No study was found on the effects of phytosterols, which are similar to cholesterol in chemical structure, on paraoxonase enzyme activity. However, there are conflicting studies showing the relationship between cholesterol and PON1 and studies on HDL in which paraoxonase is involved. Consistent with our study, Yi *et al.* showed the decreased serum PON1 activity in mice on a high cholesterol diet.[42] On the other hand, Kim *et al.* expressed that cholesterol increased PON1 activity.[43] There are also studies on the effect of phytosterol-rich foods on PON1 activity.While Sutherland *et al.* found a positive correlation between plasma phytosterol and HDL cholesterol levels, Zak *et al.* showed that phytosterol consumption increased the cholesterol level in HDL.[44,45]

Clofibrate, gemfibrozil, ciprofibrate and bezafibrate showed low affinity values. Fenofibrate showed above average affinity in normal and M/L 55 structures and below average affinity in Q/R 192 polymorphic structures. Yesilbursa *et al.* observed that fenofibrate increased PON1 activity.[46] This result contradicts our study in normal and M/L 55 constructs with above average affinity values. Macan *et al.* found that gemfibrozil significantly decreased PON1 activity.[47] Increased PON1 activity by using bezafibrate was reported by Durrington *et al.*[48] As mentioned above, the fact that fibrate-type drugs generally increase PON1 activity is consistent with the low affinity values found in our study.

Ezetimibe was in the group of drugs with high affinity for PON1. Niacin was found to have low affinity. A study identified that niacin did not affect paraoxonase and arylesterase activity, but ezetimibe decreased paraoxonase and arylesterase activity.[49] This result support our view that ezetimibe with high affinity value may have a negative effect on PON1 activity.

Sibutramine was measured as the compound with the highest affinity in the Q/R 192 polymorphic structure. As far as we have researched, there is no study showing a direct effect of sibutramine on PON1 activity. However, James et al. observed that sibutramine significantly increased plasma HDL levels. Since there were no studies showing a direct effect of sibutramine on PON1, a comparison with experimental studies could not be made.[50]

Our study has several limitations. Molecular docking analyses were performed on static protein structures and may not fully reflect dynamic interactions under physiological conditions. Additionally, computational results need to be validated in the *in vivo* environment.

These *in silico* results found in this study need to be supported by experimental studies. Although *in silico* molecular docking method saves time and money, its reliability is still a matter of debate. When the correlation between the Autodock 4 software used in this study and the affinity of PON1 with its substrates

and other experimental studies was evaluated, it was observed that the Autodock software worked well for lactone structures, but the error rate increased for compounds such as phenylacetate and very high affinity compounds. Although Autodock is one of the most widely used programs, more reliable software such as CDOCKER may be preferred in the future. Nowadays, more reliable results can be obtained by conducting molecular dynamics studies together.

In light of the findings of this study, it is evident that paraoxonase-1 (PON1) polymorphisms play a critical role in determining the interaction dynamics with lipid-lowering drugs. This highlights the importance of integrating genetic profiling into clinical practice to tailor treatment strategies effectively. Personalized medicine approaches could help optimize drug efficacy and minimize adverse effects by selecting treatments based on an individual's genetic predispositions.

Future studies should focus on validating these computational findings through in vitro and in vivo experiments to confirm their clinical relevance. Additionally, investigating the molecular mechanisms underlying PON1 interactions with a broader range of therapeutic agents could provide deeper insights into its role in personalized treatment approaches. Expanding on these findings, the development of advanced molecular dynamics simulations and large-scale genotype-phenotype correlation studies will be instrumental in bridging the gap between computational predictions and practical applications in clinical settings.

CONCLUSION

This computational study investigated the interactions between various lipid-lowering drugs and PON1, including its polymorphic forms. Our findings revealed significant variations in binding affinities, with brassicasterol, sibutramine, and stigmasterol showing the highest affinities for normal PON1, Q/R 192, and M/L 55 polymorphic structures, respectively. These results suggest that the efficacy of lipid-lowering drugs may be influenced by their interactions with PON1 and its polymorphisms, potentially impacting personalized treatment approaches. However, further *in vitro* and *in vivo* studies are necessary to validate these computational findings and establish their clinical relevance. This research demonstrates the value of *in silico* methods in exploring drug-enzyme interactions and opens new avenues for personalized medicine in cardiovascular health management.

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Authorship contribution statement

Consept and desing: ZD and BK.
Acquisition of data: ZD.
Analysis and interpretation of data: ZD, BK, AO and IY.
Drafting of the manuscript: ZD and BK.
Critical revision of the manuscript for important intellectual content: IY, AO and BK.
Statistical analysis: ZD.
Supervision: BK.

Declaration of competing interest

None of the authors have potential conflicts of interest to be disclosed.

Ethical approval

Ethical approval was not required for this study.

Availability of data and materials

Data and materials are available from the authors upon reasonable request.

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