

Insight into the interaction of human pancreatic lipase with potential anti-obesity drug, Cetilistat, using a molecular docking and molecular dynamics simulation

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Competing interests

The authors declare no conflicts of interest.

Abbreviations

Rg, radius of gyration; NWS, New World Syndrome; GAFF, Generalized Amber force field; VMD, Visual Molecular Dynamics; RMSF, root means square fluctuations; SASA, solvent accessible surface area.

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Abstract

Background: Obesity is a lifestyle disease that involves an excessive amount of body fat deposition. Cetilistat is being used to treat obesity. It mainly inhibits human pancreatic lipase, an enzyme that helps to break down the oil into small molecules of glycerol and fatty acids in the intestine. Therefore, pancreatic lipase inhibition is a potential therapeutic approach for obesity control and treatment. **Methods:** cetilistat's binding mode and interaction with human pancreatic lipase are not well understood. In this study, the human pancreatic lipase inhibitory activity of cetilistat was investigated by employing molecular docking and molecular dynamics simulation. Human pancreatic lipase has two states: closed state and open state which is controlled by a surface loop i.e. "lid region" which normally undergoes conformational changes only upon addition of lipids and then breakdown into glycerol and fatty acid. In the present study, open state conformation of the human pancreatic lipase structure was used (2OXE.pdb). The docking study reveals that the cetilistat prefers to bind at the "lid region" of pancreatic lipase. Furthermore, molecular dynamics simulation reveals that the cetilistat affects the structure and dynamics of human pancreatic lipase. Mainly, cetilistat affects the conformational changes in the "lid region" of pancreatic lipase which is important for the breakdown of lipids. Furthermore, the radius of gyration (Rg) and solvent-accessible surface area shows that the cetilistat-bound pancreatic lipase affects the compactness of the lipase structure. Thus, our computational modeling study reveals the inhibitory action of cetilistat with human pancreatic lipase and may be further useful for the design and development of anti-obesity drugs. **Results:** To explore the binding mode and interaction of HPL with cetilistat, we employed molecular docking, a molecular dynamics simulation study. The details of which are discussed below. **Conclusion:** Thus, our computational modeling study reveals the inhibitory action of cetilistat with human pancreatic lipase and may be further useful for the design and development of anti-obesity drugs.

Keywords: pancreatic lipase; Cetilistat; obesity; docking; molecular dynamics simulation

Introduction

Obesity is a state of uneven fat storage in the body that impacts human health [1]. The prevalence of obesity is rising globally now, and it has become a critical public health issue. Overweight and obesity were found to be a global epidemic condition mentioned by WHO, also depicted as “New World Syndrome” (NWS) [2]. Statistically, the unease of obesity has improved from 12%–20% in men and from 16%–25% in women over the previous ten years [3]. The mortality rate is very high due to obesity. It is the fifth leading cause of death worldwide and has reached epidemic proportions globally, with at least 2.8 million people dying each year due to being overweight or obese [2, 4]. It is also observed that obesity is an important risk factor for lifestyle diseases such as cardiovascular disease, diabetes, hypertension, and cancer [2, 5–8].

Hence, the control, prevention, and treatment of obesity further help to reduce the prevalence and mortality of such life-threatening diseases. The current obesity control drugs and surgery (liposuction) are available but show less responsible for obesity control. Hence, continuous efforts are being taken to search for potential drugs against the enzymes involved in converting oil into glycerol and fatty acid in the intestine.

In mammals, the digestion of dietary triacylglycerols is mediated by two main enzymes. The first one is a preduodenal lipase secreted in the digestive system's upper part and acts along the whole gastrointestinal tract. The second one is a pancreatic lipase that contributes to lipid digestion only in the duodenum [9, 10]. The pancreatic lipase helps to break down the oil in the food source into glycerol and fatty acids. The pancreatic lipase catalyzes the hydrolysis reaction, breaks down ester bonds of lipids and fats, and converts them into fatty acids, and glycerol [11]. Therefore, the modulation of pancreatic lipase may suggest a new insight into the discovery of several therapeutic drugs that can inhibit fat absorption in the body and control obesity. Pancreatic lipase inhibitors can make affects the decomposition ability of triglycerides into glycerol and fatty acid. They can control the fat entering the blood from the source to achieve lipid-lowering effect [12]. Hence, human pancreatic lipase inhibition is another approach to treating obesity.

Orlistat is a gastrointestinal lipase inhibitor that strongly inhibits the activities of all gastric/pancreatic lipases in vitro [13]. It is approved for use in obesity treatment and associated co-morbidities. It can be associated with undesirable gastrointestinal conditions, such as oily spotting, flatus with discharge, oily evacuation, and fecal incontinence and hence withdrawal from treatment [14–16]. In addition, cetilistat is a novel, highly lipophilic benzoxazine that inhibits pancreatic lipases [17]. The phase I clinical study reveals that cetilistat increased fecal fat excretion and was well tolerated, and dietary fat absorption was reduced in healthy volunteers [18]. Furthermore, the results of phase II, a placebo-controlled, randomized, multi-center 12-week study, show that the cetilistat treatment was well tolerated and GI adverse events, such as flatus with discharge and oily spotting, only occurred in 1.8%–2.8% of subjects in the cetilistat-treated groups [17].

Therefore, cetilistat merits further evaluation for the pharmacotherapy of obesity as well as other related disorders. However, the interaction and inhibition of human pancreatic lipase by cetilistat are not well understood at the atomic level. Hence, the mode of interaction of cetilistat with the pancreatic lipase was investigated using a molecular modeling approach.

Computational methodology

Molecular modeling of human pancreatic lipase and cetilistat

We employed a molecular modeling approach to understand the binding mode of human pancreatic lipase with the cetilistat. The crystal structure of human pancreatic lipase was retrieved from the protein database (source code: 2OXE.pdb). The missing residues of Cys256 to Asp268 were modeled using the modeler through the

Chimera interface [19, 20]. The atomic coordinates of the cetilistat were built using the Discovery Studio Visualizer [21].

Human pancreatic lipase-ligand binding site prediction

P2RANK (<https://prankweb.cz/>) webserver was utilized to predict the ligand binding sites within Human pancreatic lipase (PDB ID: 2OXE).

Molecular docking of pancreatic lipase with cetilistat

To explore the binding mode of the human pancreatic lipase with cetilistat, we performed molecular docking using the Autodock4.2 [22]. The putative binding mode and intramolecular interactions of cetilistat with human pancreatic lipase were determined by blind docking followed by local docking protocol (<http://autodock.scripps.edu>) using AutoDock4.2 [22]. The lowest binding energy conformation of cetilistat with human pancreatic lipase was further analyzed and visualized through the PyMol (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC) and Discovery Studio Visualizer 2016 (BIOVIA, Dassault Systèmes, San Diego), respectively. The least energy human pancreatic lipase and cetilistat were further used for molecular dynamics simulation to check the stability of the complex, conformational fluctuation change, etc.

Molecular dynamics simulation of pancreatic lipase with cetilistat

To explore the refined binding mode, interaction, and stability of the human pancreatic lipase with cetilistat, we employed molecular dynamics simulations using Gromacs2020.5 [23]. The amber ff99SB force field parameters were applied for the human pancreatic lipase. The Generalized Amber force field (GAFF) parameters were generated for the cetilistat using the AM1-BCC charges through the “Antechamber” module of AmberTools18, similar to earlier work [24, 25]. The HPL-cetilistat complexes were solvated using a TIP3P water module in a cubic box of 10 Å, and the system was neutralized by using the counter ions. The “Xleap” module of AmberTools18 was used to prepare the topology and coordinate files. The “Parmd tool” was used to convert the Amber-generated files into the gromacs companionable files [26, 27]. Next, energy minimization was performed using the steepest descent method followed by the conjugate gradient method. Then, the system was equilibrated using the NVT, followed by NPT dynamics simulations for 500 ps. Finally, a production MD simulation of 100 ns was performed for HPL and HPL-cetilistat complex. All other simulation parameters are similar to an earlier study [28, 29]. Visual Molecular Dynamics (VMD) and PyMol were further used to visualize and analysis of the trajectory [30, 31].

Results and discussion

To explore the binding mode and interaction of HPL with cetilistat, we employed molecular docking, a molecular dynamics simulation study. The details of which are discussed below; Ligand binding site residues of human pancreatic lipase. The Table (1) represents the ligand binding sites within human pancreatic lipase.

Molecular docking

To explore the interaction of human pancreatic lipase with the cetilistat, we employed molecular docking using AutoDock4.2 (Morris et al., 2009). The least binding energy conformation of cetilistat was found to be -7.20 kcal/mol, as shown in Figure 1. The least binding energy structure of cetilistat was found near the “lid region” of pancreatic lipase which is important for the breakdown of lipids, as shown in Figure 1. The human pancreatic lipase and cetilistat complex forms hydrogen bonding interaction with Gly261 (2.1 Å) and Phe262 (2.6 Å), as shown in Figure 1. In addition, Gly218 forms carbon-hydrogen bonding interactions with cetilistat, Tyr218, and Phe219 forms π - π T-shaped interactions, and Phe81 forms π -alkyl type of interaction, Lys236, Leu217 forms alkyl type of non-bonded interaction with cetilistat as shown in Figure 1B. The docking analysis reveals that the cetilistat prefer the binding site near the lid region of

Table 1 The ligand binding sites within human pancreatic lipase

| NO | Probability | Chain ID_residue number |
|----------|-------------|--|
| Pocket 1 | 0.044 | B_170, B_171, B_277, B_282, B_283, B_95, B_96, B_98 |
| Pocket 2 | 0.043 | A_225, A_237, A_239, A_246, A_249, A_251, A_305, A_306, A_307, A_345 |
| Pocket 3 | 0.034 | B_225, B_237, B_239, B_246, B_249, B_251, B_305, B_306, B_307, B_345 |
| Pocket 4 | 0.026 | B_133, B_199, B_234, B_274, B_275, B_277, B_96 |
| Pocket 5 | 0.019 | A_146, A_149, A_25, A_49, A_54, A_56, A_71, A_72, A_73 |
| Pocket 6 | 0.015 | A_133, A_134, A_199, A_234, A_273, A_277, A_96 |
| Pocket 7 | 0.004 | A_170, A_171, A_277, A_282, A_96 |

human pancreatic lipase (Figure 1), the open and closed conformation of pancreatic lipase is essential for the breakdown of lipid, and the cetilistat might affect these conformational changes, as it shows significant affinity with the pancreatic lipase.

Molecular dynamics simulation

Root means square deviation

To investigate the binding mode and stability of human pancreatic lipase with Cetilistat, we employed molecular dynamics simulation for 100 ns using Gromacs 2020.5 [23]. The stability of the MD simulated system was assessed using the root mean square deviations of C α backbone atoms for HPL and HPL-Cetilistat complexes (Figure 1). The RMSD plot reveals that the cetilistat affects the conformational dynamics of the human pancreatic lipase, as shown in Figure 1. It is also observed that the cetilistat forms a stable complex with HPL (Supplementary Movie 1), which further leads to reducing conformational dynamics of HPL. Here, HPL "lid region" (Figure 2) shows reduced fluctuations compared to HPL without a drug complex (Supplementary Movie 1). The MD simulation results reveal that the cetilistat affects the HPL conformational property mainly in the "lid region", which is important for the open state > closed state conformation of pancreatic lipase and further breakdown of lipids (Supplementary Movie 1). Moreover, to explore the effect of cetilistat binding on the conformational flexibility of HPL, root means square fluctuations (RMSF) were calculated and shown in Figure 2.

The RMSF plot provides information on the flexible and constrained regions of the MD simulated system by calculating the degree of movement of C α atoms around their average positions. The higher RMSF value shows a flexible region, and the low RMSF value shows the constrained regions, as shown in Figure 2. The RMSF plot shows that cetilistat affects the conformational dynamics of HPL at the "lid region", which is essential for its function. Hence, understanding the structural compactness of both the system viz., HPL and HPL with cetilistat, the Rg and solvent accessible surface area (SASA) was calculated summarised below.

Pancreatic lipase compactness

The Rg value indicates the simulated system's compactness level to obtain a structural insight into the stability [32]. The analysis of the Rg value (Figure 3A) reveals that HPL has a higher Rg value than the HPL-Cetilistat complex (Figure 3A). The Rg graph also shows the compact and stable behavior of the HPL-Cetilistat complex, while the HPL without Cetilistat shows higher conformational fluctuations. This indicates that the cetilistat forms a stable complex and affects the structural dynamics, which is important for HPL function. Furthermore, the SASA (Figure 4) also agrees with the Rg data. Here, the SASA graph shows that the cetilistat reduces the structural compactness and conformational fluctuations in the HPL. Here, the HPL-Cetilistat complex shows a lower SASA value than the HPL (Figure 4).

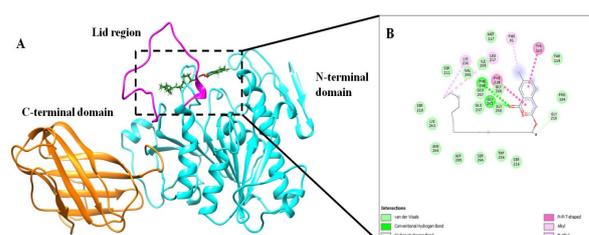


Figure 1 Binding mode of Cetilistat with human pancreatic lipase using molecular docking. Analysis of MD simulated structure of pancreatic lipase and cetilistat.

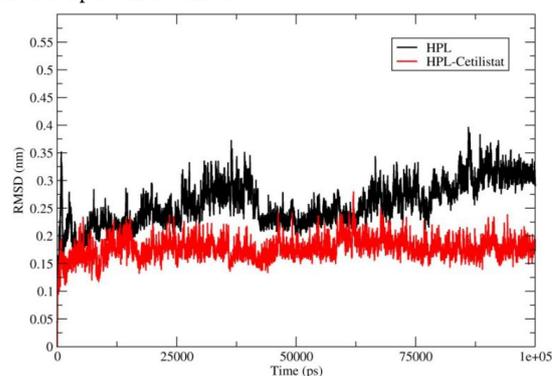


Figure 2 Root means square deviations (RMSD) of human pancreatic lipase. Here, human pancreatic lipase is shown in black, and human pancreatic lipase with cetilistat is shown in red color for 100 ns MD simulation. The plot shows that cetilistat affects the conformational dynamics of human pancreatic lipase. RMSD, Root means square deviations.

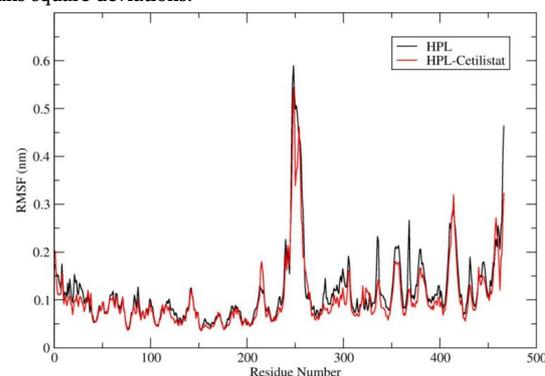


Figure 3 Root mean square fluctuation of Human pancreatic lipase. Here, human pancreatic lipase is shown in black, and human pancreatic lipase complex with cetilistat is shown in red color for 100 ns MD simulation. The RMSF plot reveals that cetilistat reduces the structure and dynamics of the HPL.

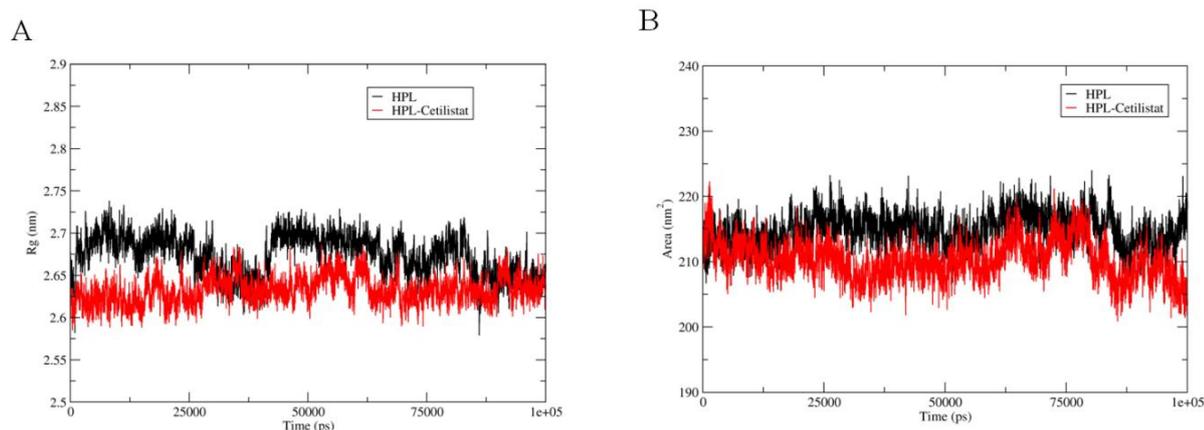


Figure 4 The radius of gyration (Rg) and Solvent accessible surface area (SASA) of Human pancreatic lipase. Here, (A) shows the Rg plot and (B) shows the SASA plot of human pancreatic lipase (black) and human pancreatic lipase complex with cetilistat (red) for 100 ns MD simulation. Rg, radius of gyration; SASA, Solvent accessible surface area.

Analysis of MD simulated structure of pancreatic lipase and cetilistat

To understand the refined binding mode of interactions of human pancreatic lipase with the cetilistat, MD simulated 100 ns end structure was analyzed as shown in Figure 4. The analysis of the MD simulated structure shows that the cetilistat is stabilized by the hydrogen bonding interactions with Ser216 (2.1 Å) and Gly261 (2.6 Å), carbon-hydrogen bonding interactions with Ser216 (2.5 Å) and Glu257 (2.8 Å) as shown in Figure 4B. In addition, Phe219 forms π - π stacked interaction, ILE259 Amide- π stacked interaction, Tyr118 forms π -alkyl type of interaction, and Pro184 forms alkyl type of interactions with cetilistat. Ley271, Lys242, Asp251, Lys243, ILE252, Trp256, Gly260, and Ser264 forms van der Waals interactions with cetilistat.

The MD simulated end structure analysis reveals that the cetilistat is stable at the binding site and forms bonded and nonbonded interactions with pancreatic lipase. The MD simulated structure shows the hydrogen bonding interaction of Gly261 with cetilistat (Figure 4B), similar to the pancreatic lipase and cetilistat docked complex (Figure 1B).

Conclusion

In the present study, molecular docking and molecular dynamics simulations were employed to investigate human pancreatic lipase's binding mode and inhibitory mechanism with cetilistat. It is revealed that the cetilistat prefers to bind at the "lid region" of human pancreatic lipase. Further, molecular dynamics simulation shows stable binding of cetilistat with pancreatic lipase further affects structure and dynamics. MD simulation study reveals that the pancreatic lipase forms bonded and nonbonded types of interactions to stabilize the cetilistat. Furthermore, the function of pancreatic lipase is dependent upon the conformational changes in the "lid region" for binding of triglycerides and breakdown into glycerol and fatty acid, and the binding of cetilistat at the "lid region" affects its conformational transition as revealed from molecular dynamics simulation (Supplementary Movie 1). Hence, our computational modeling study could help to understand the binding mode and interaction of cetilistat with human pancreatic lipase and could further help to design a potential cetilistat analog for obesity management and treatment.

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