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Pancreatic Lipase Inhibition by Papaverine: Investigation by Simulated Molecular Docking and Subsequent In Vitro Evaluation

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ABSTRACT

In order to develop safe and effective lipid lowering drug that affecting the absorption of dietary lipids, the pancreatic lipase inhibitory effect of papaverine alkaloid was probed. The investigation included molecular docking to fit papaverine into the binding site of pancreatic lipase employing optimal set of parameters succeeded in retrieving the closest model to the cocrystallized pose. Docking simulation suggested four binding modes for papaverine. The highest ranking binding mode have potential hydrophobic interactions with the key amino acids Phe-215, Ala-178, Pro-180 and Ala-259 and potential aromatic stacking between isoquinoline ring and Phe-77 and Tyr-114. Moreover, papaverine forms strong hydrogen bonds with the key amino acid Ser-152 in the catalytic triad. Experimentally, papaverine illustrated substantial in vitro inhibitory effect against PL with IC_{50} = 36.2 µg/ml (106.6 µM).

Keywords: Papaverine; Pancreatic Lipase; Obesity; Docking Simulations; Enzyme Inhibition.

INTRODUCTION

Obesity is a worldwide rapidly growing epidemic condition, presenting an increase in the risk of mortality and morbidity in many countries across the world.[1] Currently, 1.4 billion adults are overweight. Of these over 200 million men and nearly 300 million women were obese.[2] The World Health Organization (WHO) defines obesity as an abnormal or excessive fat accumulation harmful to human health. Several complications are associated with obesity, such as hyperlipidemia, hypertension, diabetes mellitus, cardiovascular disease and cancer. These serious disorders force researchers to find long-term solutions for weight management and control .[3,4]

Several strategies were adopted for combating obesity. Nonpharmacological approaches to lose weight such as reduction of calorie intake and increased level of physical activity are very well-known, however, the needs for drugs are still required. A lot of diets regime have been recommended for weight loss but there is little scientific evidence to recommend one diet over another.[5] As a result of the inconsistent effort in achieving a negative energy balance through diet and exercise, the need for drugs and other supplements are fast gaining acceptance. Several types of antiobesity drugs were developed and some were available in the market.[6] One of these is orlistat, which decreases intestinal absorption of fat via inhibition of pancreatic lipase (PL).[7] Others such as sibutramine, an appetite suppressant, has been associated with increased cardiovascular events and strokes and has been withdrawn from the market in several countries.[8]

At present, because of dissatisfaction with high costs and potentially dangerous side-effects, the potential of natural products for treating obesity is under exploration, and this may be an excellent alternative strategy for developing

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future efficient, safe lipid lowering agents.[9-11] Natural products are considered important sources for new drugs or lead compounds.[12] The structural diversity of natural products combined with the fact that they are synthesized within living systems render them more "drug-like" than totally synthetic molecules.[13,14] A variety of natural products, including crude extracts and isolated compounds from plants, were found to have a body weight reduction effect and could reduce diet-induced obesity. Therefore, they have been used in treating obesity.[15] Among treatments for obesity, one of the most talented strategies to reduce energy intake through gastrointestinal mechanisms, without affecting the central mechanisms, is the development of absorption inhibitors.[16] Fats in diet are not absorbed from the intestine directly unless being digested by pancreatic lipase. Therefore, pancreatic lipase is one of the most widely investigated mechanisms for determining the potential efficacy of different compounds as anti-obesity agents.[16] Pancreatic lipase hydrolyzes triglycerides to monoacylglycerols and fatty acids. The tetrahydrolipstatin (orlistat) is an active site-directed inhibitor that interacts with the key nucleophilic serine residue from the catalytic triad of pancreatic lipase.[17] Although this pancreatic lipase inhibitor is clinically approved for obesity treatment, it has gastrointestinal side-effects.[18] Therefore, researchers are screening novel inhibitors, derived from plants or other natural sources, that lack some of these unpleasant side-effects. Natural products provide a huge pool for the discovery pancreatic lipase inhibitors. Therefore, screening and optimization of safe and effective anti-obesity phytochemicals would provide an excellent new strategy in combating obesity with its complications. Recently, we have established a screening research for plant extracts and natural phytochemicals in order to discover PL inhibitors.[9,19,20] In the course of a search for natural PL inhibitors from herbal medicines[18], papaverine was chosen for more detailed investigation, since the alkaloid has a privileged scaffold and structurally similar to several natural pancreatic lipase inhibitors (e.g. 3,3′,4,4′-tetrahydroxy-2-methoxychalcone, IC_{50}= 35.5 ± 0.5 µM, Fig.1).[21]

Papaverine (Fig. 1A) is a prominent member of isoquinoline alkaloids isolated from opium poppy (*Papaver somniferum* L.).[22] The alkaloid has non-selective smooth muscle relaxant effects, therefore it is used clinically in the treatment of vasospasmic diseases.[23,24] Papaverine increases the unidirectional K influx into muscles causing vasodilatation and muscle relaxant effects.

The present study started by molecular docking simulations for papaverine into the binding pocket of PL in order to reach to a preliminary evidence about alkaloid/PL binding energetics. Finally, papaverine was tested in vitro against PL to evaluate its inhibition potential.

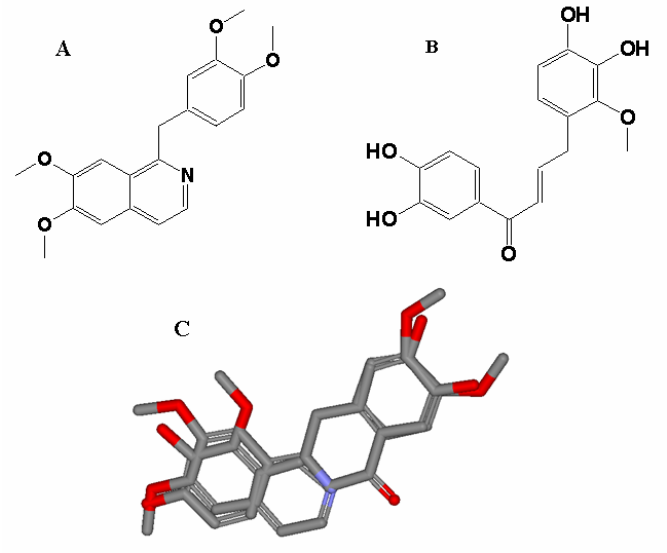

Figure 1: (A) Structure of Papaverine, (B) 3,3′,4,4′-tetrahydroxy-2-methoxychalcone, (C) Overlaid structures of papaverine and the chalcone derivative.

RESULTS AND DISCUSSION

Natural compounds are specially adapted for their interactions with biological systems as they created by mother nature,. Therefore, they are considered as valuable sources for drug discovery. Over 70% of New Chemical Entities (NCEs) introduced into the market between 1981-2006 were natural products or derived or developed from natural products.[25] Since natural compounds exhibit pleiotropic action and could bind to multiple targets,[26] therefore phytochemicals identified from traditional medicinal plants represent good opportunity for the development of newer anti-obesity lipase inhibitors. As a part of our screening project for biologically active anti-obesity agents from natural resources, papaverine has been investigated for its anti-lipase activity. Papaverine has isoquinoline scaffold, which is an important structural motif commonly encountered in natural alkaloids of interesting biological profiles. In particular, this framework has become widely identified as "privileged" structure or pharmacophore that can bind to multiple target proteins.[27] This fact combined with the similarity between papaverine and chalcone-based PL inhibitors scaffold (e.g., compound **B** Fig.1),[21] has prompted us to evaluate the potential anti-lipase activity of papaverine. Our selection is based on a simple assumption that two chemical compounds of similar chemical structure could have similar biological activity. The use of structural similarity to help in the prediction of biological activity of natural compounds is a valid and applicable strategy.[28] The investigation commenced by evaluating the possibility of binding via computer-aided molecular modeling techniques. Accordingly, papaverine was docked into the binding pocket of PL (1LPB). The binding site was defined from the crystallographic structure of a bound high-affinity ligand (CllP, Figure 2, see Docking Simulations under Methods). The docking study was conducted utilizing the docking engine FRED.[31] FRED docks molecules employing a comprehensive search algorithm. It systematically searches rotations and translations of each conformer of the ligand within the active site and filters unrealistic poses. Pose generation is followed by estimating the strength of ligand-target interactions (scoring). The final docked conformations (poses) are ranked according to their scores. However, molecular docking requires the user to provide FRED with an optimal set of parameters for the simulation experiment. In order to identify the correct parameters, self-docking strategy were adopted in this work. Accordingly, the docking/scoring conditions which retrieve the closest model to the crystallographic structure were employed in the docking simulation experiment.[9] Docking simulations suggested four binding modes for papaverine within PL. The lower pose-entropy obtained in our docking simulation experiment was because of the rigidity of papaverine structure. The molecular interactions of the highest ranking binding mode can be summarized in figure 3. Obviously from figure 3A, papaverine fits well within the binding site of PL. Upon comparing the docked pose with the highest score (Fig.3b) with the co-crystallized ligand within the binding site of PL (Fig.3c) we can notice similarities in their binding profiles. Both have potential hydrophobic interactions with the amino acids Phe-215, Ala-178, Phe-77 and Tyr-114. However, papaverine has a further potential hydrophobic interactions with Pro-180 and Ala-259 and potential aromatic π–π stacking between isoquinoline ring and Phe-77 and Tyr-114. Moreover, the nitrogen atom forms a potential strong hydrogen bond with Ser-152, which stabilizes the ligand-protein complex and could contribute to the affinity of papaverine. In the same way, the co-crystallized ligand has analogous interactions with Ser-152 (Fig.3c). However, it is strongly hydrogen bonded with three more amino acids; Leu-135, His-263 and Phe-77. In the structure of PL, His-263, Asp-176 and Ser-152 form a catalytic triad representing the lipolytic site. Moreover, enzymatic activity has shown to be diminished after chemical modification of Ser-152 indicating its essential role for the catalytic activity.[29] Therefore, it is unsurprising that compounds strongly bind to the catalytic triad; especially Ser-152 could inhibit the lipolytic activity. The proposed inhibitory action of papaverine was experimentally validated against porcine pancreatic lipase type II using the *p*-nitrophenylbutyrate (PNPB) as substrate. The enzymatic

reaction progression was monitored through the release of *p*-nitrophenol. The in vitro activity was expressed as the concentration of papaverine that inhibited enzyme activity by 50% (IC_{50}), which was found to be 36.2 µg/ml (106.6 µM, Fig.4).

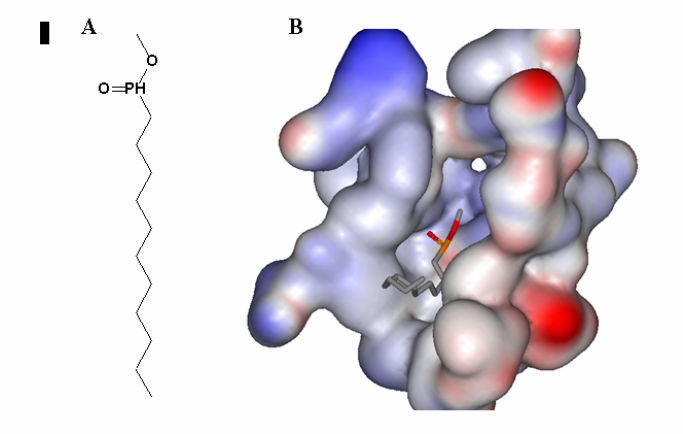

Figure 2: (A) Structure of ClIP PL inhibitor. (B) The solvent accessible surface area of the binding site of PL (1LPB) and the co-crystallized inhibitor (ClIP).

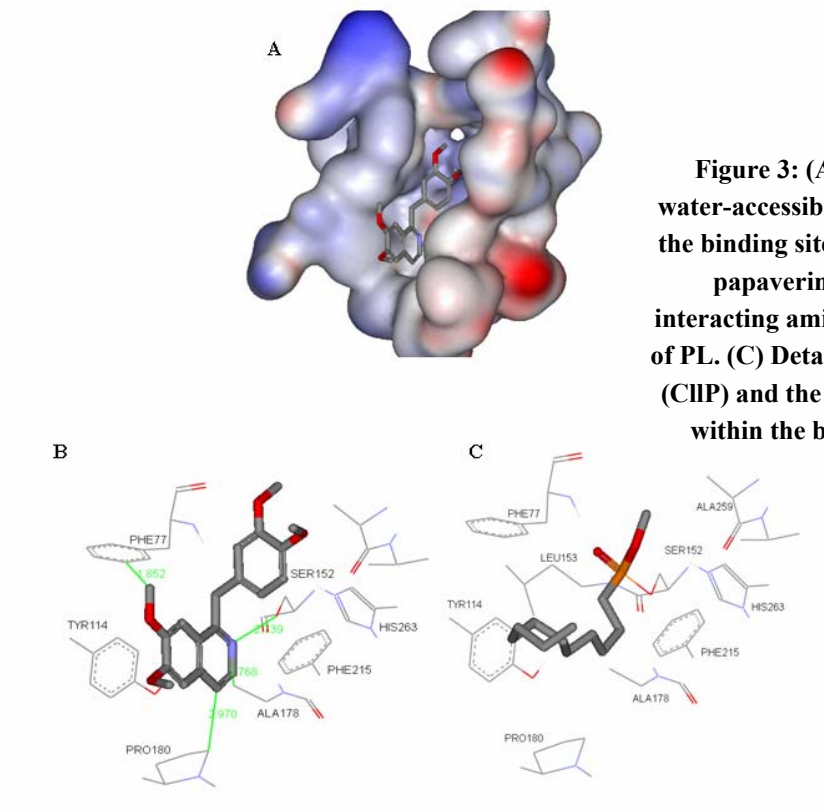

Figure 3: (A) Papaverine structure docked into the water-accessible surface (probe diameter 1.4Å) within the binding site of PL. (B) Detailed view of the docked papaverine structure and the corresponding interacting amino-acid moieties within the binding site of PL. (C) Detailed view of the co-crystallized structure (ClIP) and the corresponding interacting amino-acids within the binding site of PL (PDB code: 1LPB).

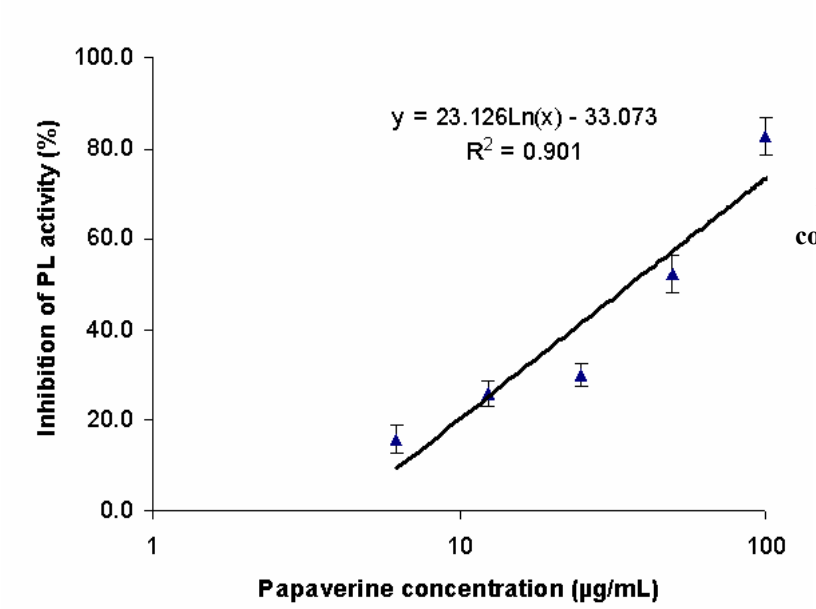

Figure 4: The effect of variable papaverine concentrations on the activity of pancreatic lipase (PL).

CONCLUSION

Molecular docking simulations demonstrated clearly that papaverine can readily dock within the binding pocket of PL in a low energy orientation with multiple attractive interactions with key amino acids including Ser-152 which is part of the catalytic triad of the enzyme. Further experimental testing against porcine pancreatic lipase type II, using the *p*-nitrophenylbutyrate (PNPB) as substrate, unequivocally proved that papaverine inhibits PL activity (IC_{50} = 36.2 µg/ml). Papaverine could be used as a useful lead compound for optimization into more potent and selective PL inhibitors as anti-obesity agents. Moreover, the experimental anti-PL activity of papaverine increases the confidence in our model for predicting the anti-PL of closely related natural compounds.

EXPERIMENTAL

Materials

All of the chemicals used in these experiments were of reagent grade obtained from the following sources: Porcine pancreatic lipase type II; tris-HCl buffer; Orlistat; Papaverine and p-nitrophenylbutyrate (PNPB) from Sigma (USA); acetonitrile and methanol from Merck (USA).

Molecular modeling

Software and hardware: The following software packages were utilized in the present research:

- CS ChemDraw Ultra 7.01, Cambridge Soft Corp. (http://www.cambridgesoft.Com), USA.
- OMEGA (Version 2.3.2), OpenEye Scientific Software (www. eyesopen.com), USA.[30]
- FRED (Version 2.2.5), OpenEye Scientific Software, (www.eyesopen.com), USA.[31]
- DS visualizer 2.0, Accelrsy Inc. (www.accelrys.com), USA.

Docking Simulations

The chemical structure of Papaverine (Fig. 1) was sketched in ChemDraw Ultra (7.01) and saved in MDL molfile format. Subsequently, a group of energetically accessible conformers was generated using OMEGA2 software.[30] OMEGA rapidly generates conformational ensembles of small molecules using a fragment-based library in order to build initial models of structures by assembling these fragment templates followed by rule-based torsion search stage. The generated conformers are saved in SD format. The 3D geometry of PL were obtained from the Protein Data Bank (PDB code: 1LPB,

resolution; 2.46 Å).[32] Hydrogen atoms were added to the protein using the DS visualizer. The docking study was conducted in the presence of explicit water molecules. The drug was docked into the binding site of PL employing FRED software.[31] The ligand conformers and protein structure are treated as rigid during the docking process. FRED docking roughly consists of 2 steps: shape fitting and optimization. Firstly, the ligand is placed into a grid box including all active-site atoms (fitting phase) thereafter, a series of three optimization filters are employed (optimization phase). The filters include: refining the position of hydroxyl hydrogen atoms of the ligand; rigid body optimization, and finally optimization of the ligand pose in the dihedral angle space.[31] The input files for FRED docking include: the conformers of papaverine generated using OMEGA software; the target protein structure (1LPB.pdb); a box defining the active site based on the co-crystallized ligand (expanded by 2Å); and several optional parameters. Docking settings that succeeded in reproducing the experimental pose of the co-crystallized ligand (CllP, for more details see reference 9] were employed here. The docked poses were scored by the Chemgauss2 scoring function and the highest ranking poses were retained for evaluation. Hydrogen bonding interactions are the most significant chemical potentials accounted for by the Chemgauss2 scoring function. The Chemgauss2 function is the sum of the following potentials, 1) Shape based interactions between all heavy atoms. 2) Hydrogen bonding interactions based on favorable interactions between polar hydrogens and lone pairs. 3) Aromatic ring interactions based on favorable interactions between aromatic atoms and the π-electron positions plus repulsive aromatic-atom to aromatic atom and π-electron to π-electron interactions.[31]

Pancreatic Lipase inhibition assay

A stock solution of papaverine was initially prepared in DMSO. Subsequently, 20 µL of this solution was used to give final concentration range of 2.0–100 µg/mL in the reaction mixture. The PL activity was evaluated by a colorimetric assay that measures the release of p-nitrophenol as previously described [9]. Crude porcine pancreatic lipase type II was suspended in tris-HCl buffer (2.5 mmol, pH 7.4 with 2.5 mmol NaCl) to give a concentration of 200 unit/mL and mixed using a stirrer for 15 min. Then, the solution was centrifuged at 1500 g for 10 min and the clear supernatant was obtained. A 0.10 mL of PL solution was mixed with different concentrations (2.0–100 µg/mL) of papaverine for 5 min at 37 °C then the PNPB substrate (10 mM in acetonitrile) was added. The volume was completed to 1 mL using the tris-HCl buffer before measuring the solution absorbance spectrophotometrically at 410 nm at least 5 time points: 1-5 min. The increase in absorbance at 410 nm against blank using denaturated enzyme was measured. The PL activity is related to the rate of p-nitrophenol release which can be estimated from the slope of the linear segment of absorbance vs time profiles. The final concentration of DMSO was fixed and did not exceed 2.0%. The percentage of residual activity of PL was determined by comparing the lipase activity of PL with and without the compound. Orlistat was used as a positive control in the assay mixture.

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Declaration of interest: The author reports no conflicts of interest.

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تثبيط خميرة لايبيز البنكرياس بوساطة الببفيرين: استكشاف عن طريق محاكاة الإرساء الجزيئي متبوعا بفحص مختبري لتقييم الفعالية

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ملخـص

من أجل تطوير دواء مضاد للسمنة من خلال التأثير المثبط لامتصاص الدهون الغذائية تم استكشاف التأثير المثبط لمركب الببفرين الألكلويدي على خميرة لايبيز البنكرياس. وقد شمل البحث تجارب محاكاة الإرساء (simulated docking) لإرساء الببفرين داخل جيب الارتباط لخميرة لايبيز البنكرياس، وقد وجد أن الببفرين يرسو بسهولة وبشكل فراغي منخفض الطاقة في داخل جيب الارتباط، مثبتا نفسه بمجموعة من قوى التجاذب مع أحماض أمينية أساسية. تجريبيا، وجد أن الببفرين يثبط خميرة لايبيز البنكرياس بشكل كبير بقيمة IC_{50} = 36.2 µg/ml (106.6 µM).

الكلمـات الدالـة: ببفرين، خميرة لايبيز البنكرياس، السمنة، محاكاة الإرساء، تثبيط انزيمي.

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