Screening of lipid-lowering chemical constituents in Shuang Dan capsule based on target protein

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Abstract: Shuang Dan capsule is widely used in the market to reduce blood lipids. Literature studies have shown that its active ingredients Salvia miltiorrhiza, Cortex moutan can play a role in clearing heat and cooling blood, promoting blood circulation and removing blood stasis, but the pharmacological basis of Salvia miltiorrhiza and Cortex moutan and the mechanism of action has not been clarified. The study used molecular docking technology to virtually screen the main active small molecule compounds in Salvia miltiorrhiza and Cortex moutan, and selected cholesterol ester transfer protein (4F2A), human pancreatic lipase (1LPB) and HMG-CoA reductase (1HWI) as target proteins. Using 0SF, BOG and 115 as ligand small molecules, molecular docking, screening small molecules with compounding score higher than the threshold and binding ability, analyzing the interaction between the components and the target, and finally determining the highest score and binding energy. The lowest of the three classes of small molecules, they may be the most effective component of blood lipids in Shuang Dan capsule. Through the molecular docking technology, the understanding of the mechanism of Shuang Dan capsule's blood lipid lowering provides a theoretical basis for the structural transformation and deep research and development of hypolipidemic drugs.

1. Research background

Hyperlipidemia, that is, high blood lipid levels in humans, means that the content of one or more lipids or lipoproteins in human blood exceeds the prescribed limit, which can directly cause diseases that seriously endanger human health, such as atherosclerosis and crown. Heart disease, pancreatitis, etc. In recent years, the incidence of hyperlipidemia has been rising, and it has been called one of the three major killers of human health [1]. According to research, the cause of hyperlipidemia-induced disease is mainly related to the activities of three proteins: cholesterol ester transfer protein (4F2A), human pancreatic lipase (1LPB), and HMG-CoA reductase (1HWI).

Shuang Dan capsule consists of Salvia miltiorrhiza and Cortex moutan 2 traditional Chinese medicines. The main active ingredients include phenolic acids [2], Salvia miltiorrhiza ketones, Paeoniflorin, Paeonol, etc. [3]. According to the records of traditional Chinese medicine, Salvia miltiorrhiza and Cortex moutan are required to effectively reduce blood lipids, prevent and treat cardiovascular and cerebrovascular diseases such as atherosclerosis and coronary heart disease [4]. However, the mechanism of its action is still unclear, and the research space based on the use of traditional Chinese medicine to reduce blood lipids needs to be further improved.

The key to the clinical efficacy of Chinese herbal medicine is that its effective chemical composition binds to the target of the corresponding receptor protein and exerts its pharmacological action. It forms drugs through different types of forces such as electrostatic interaction, ionic bonding, hydrogen bonding and van der Waals force. Body complex, thereby reducing the activity of the target protein, to achieve inhibition [5]. Molecular docking technology is a method for drug design through

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the characteristics of receptors and the interaction between receptors and drug molecules [6]. It is a major technology in the field of computer-aided drug research in recent years. Therefore, this study used molecular simulation software to set the lowest molecular weight of the ligands corresponding to the target protein in the market or in the clinical stage [7], combined with the virtual screening technology for the target compounds in Salvia miltiorrhiza, Cortex moutan The activity analysis, based on the ADME/T principle, predicts the pharmacokinetic characteristics and toxicological characteristics of the target small molecule with a score above the threshold, and further selects the small molecule compound that meets the requirements and has the highest score for energy matching and spatial matching. Mutual recognition to form molecular compounds, and predict the structure of the complex and the interaction of its active sites, preliminary analysis of the mechanism of action of the main components of Shuang Dan capsule to reduce blood lipids, provide a reference for the clinical application of the drug, and help Further develop the Chinese herbal medicine component with stronger blood lipid lowering effect [8].

2. Materials and Methods

2.1 Target protein acquisition and processing

The three-dimensional crystal structure of cholesterol ester transfer protein (4F2A), human pancreatic lipase (1LPB), and HMG-CoA reductase (1HWI) was downloaded from the protein database RCSB (https://www.rcsb.org) and used as Target protein; pretreatment of the original structure, hydrogenation, charge operation, removal of water molecules, and energy optimization. The sites where 0SF, BOG and 115 are located respectively serve as active sites of the ligand small molecule.

2.2 Preparation of ligand small molecules

The related active compounds of Salvia miltiorrhiza and Cortex moutan were searched from the Chinese Medicine Database (http://tcm.cmu.edu.tw) to establish a database of molecular components of a total of 157 active components. Download the 3D structure of small molecule chemical components from PubChem (http://pubchem.ncbi.nlm.nih.gov) and convert them into three-dimensional structure images by Pymol software, hydro treating each molecule and optimizing the Minimize energy. It is converted into flexible molecules for docking using Flexible Alignment.

2.3 Molecular docking

The selected molecules were virtually screened using Autodock molecular simulation software and the return energy values of several ligand conformations were obtained. Set the grid size to 60×60×60 and the grid spacing to 0.375 Å. Using the default genetic algorithm, the maximum number of escapes is 2,500,000 and the number of runs is set to 100. The chiral carbon, the stereochemistry of the hydrogen bond acceptor, the absence or presence of hydrogen, the substructure and superstructure, the number of rings, the rotatable bonds, and the donor group are then examined using the Pymol software. All molecules were statistically sorted according to the scoring results, and the molecules with binding energies below -7.0 kcal/mol were determined by screening [9].

3. Results and discussion

3.1 Prediction of pharmacokinetic characteristics and toxicity

ADME/T refers to the pharmacokinetic method of absorption, distribution, metabolism, excretion and toxicity of drugs. It can be used for drug evaluation and screening of drug-receptor complexes. It is a commonly used detection method in drug screening and design [10]. In this study, 157 Salvia miltiorrhiza, Cortex moutan target active molecules were analyzed by ADME/T pharmacokinetic method, and rational reference to the pharmacokinetic characteristics of the

compound molecules, including blood-brain barrier permeability (BBB), human intestinal absorption (HIA), plasma protein binding rate (PPB), oil-water partition coefficient (logPo/w), topological molecular polar surface area (TPSA), five parameters [11]. The limits are as follows in Table 1.

Table 1. ADME/T parameter suggested screening criteria

Parameter	Screening criteria
Blood Brain Barrier Permeability (BBB)	≤-0.3 (non-penetrating) -0.3 to 0.3 (medium penetration) ≤0.3 (high penetration)
Human intestinal absorption (HIA)	As the case may be
Plasma protein binding rate (PPB)	Binding is< 90% (No markers flagged and AlogP 98< 4.0) Binding is> 90% (flagged at 90% or AlogP 98> 4.0) Binding is> 95% (flagged at 95% or AlogP 98> 5.0)
Oil-water partition coefficient (logPo/w)	<4.15
Topological Molecular Polar Surface Area (TPSA)	≤6×10-19m^2 (penetrating cell membrane)

According to the predicted results, six small molecular compounds above the target threshold and in line with the ADME/T pharmacokinetic method were finally obtained. The pharmacokinetic parameters are shown in Table 2 below:

Table 2. Pharmacokinetic parameters of 6 molecules

No	Compounds	BBB	HIA	PPB	logPo/w	TPSA
1	Salvianolic Acid D	0.02	32.30	74.13	-0.70	137.01
2	Salvianolic Acid C.	0.04	68.20	94.69	0.66	180.71
3	Dihydrovalepotriate	0.09	93.04	76.71	3.50	100.68
4	Daphne lone	1.97	92.87	89.34	2.93	57.53
5	Suffruticosol A1	0.25	95.86	75.39	2.53	37.81
6	Neocryptotanshinone	0.44	93.06	96.44	3.06	74.60

3.2 Molecular docking

Based on this theory, based on the selected 6 target molecular compounds, three types of small molecules Salvianolic Acid D and Salvianolic Acid C with binding energies of -7.87kcal/mol, -7.90kcal/mol, and -7.72kcal/mol were selected. Neocryptotanshinone is molecularly docked with cholesterol ester transfer protein (4F2A), human pancreatic lipase (1LPB), and HMG-CoA reductase (1HWI). These three types of small molecules have the greatest potential activity and may become the most effective lipid-lowering component in Shuang Dan capsule. The docking conformation is shown in Figure 1-3.

The Salvianolic Acid D was designed to be molecularly docked with cholesterol ester transfer protein (4F2A) (Fig. 1). The amino acid residues bound to the target protein by the target small molecule were Ala195, Val198, Gln199, Ala202, Ile215, Jeu26, Cys13, Tyr262, Gly10, Arg14, Leu217, Ser230 and Gly134.

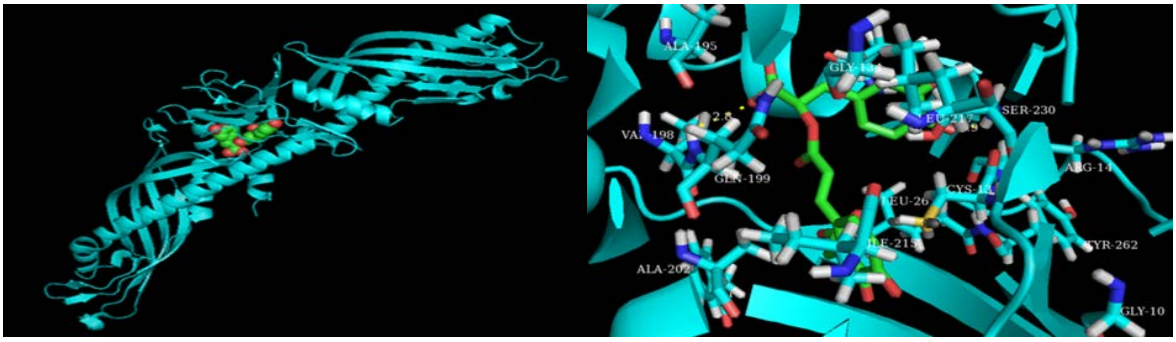


Figure 1. Confluent conformation diagram of Salvianolic Acid D and target protein cholesterol ester transfer protein (4F2A)

The silencing profile of Salvianolic Acid C and the target protein human pancreatic lipase (1LPB) was analyzed (Fig. 2). Salvianolic Acid C and the protein receptor structure were 7 of Ile248, Asp247, Ala332, Asp331, Arg337, Asp389 and Lys367. There are interactions between amino acid residues.

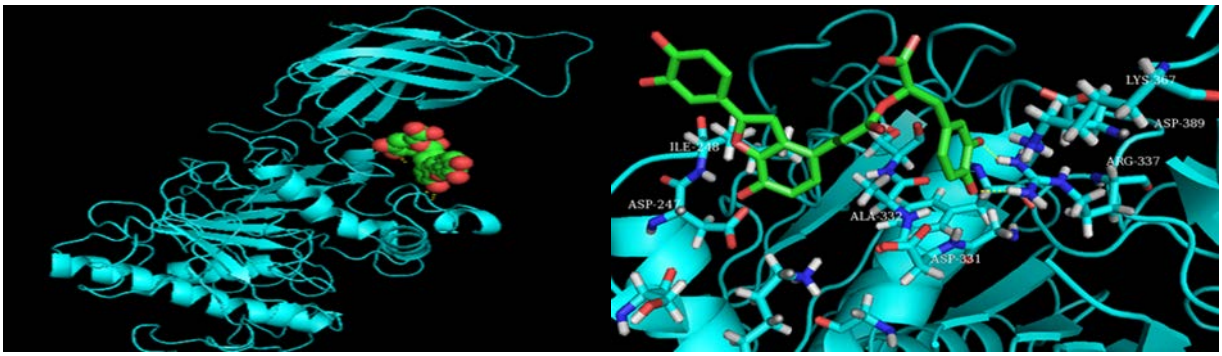


Figure 2. Confluent conformation diagram of Salvianolic Acid C and target protein human pancreatic lipase (1LPB)

According to the conformational map of Neocryptotanshinone and the target protein HMG-CoA reductase (1HWI) (Fig. 3), the catalytic active sites involved in lipid lowering include: Ser56, Gys561, Leu562, Gly849, Glu559, Asn755, His752, Ala751, Lys735, Leu857, Leu853.

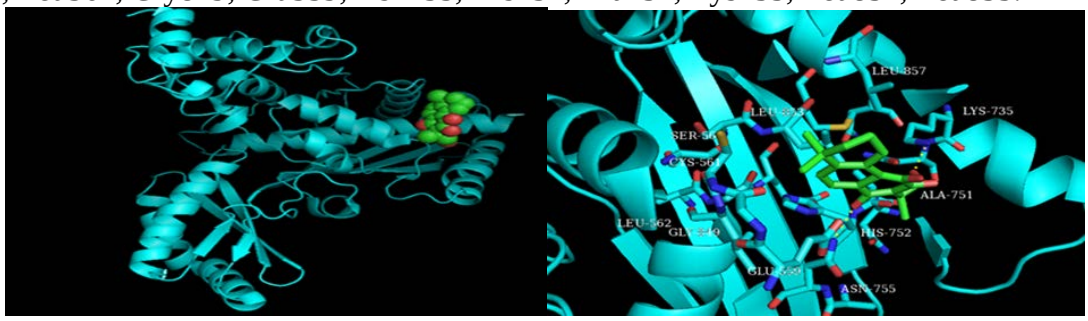
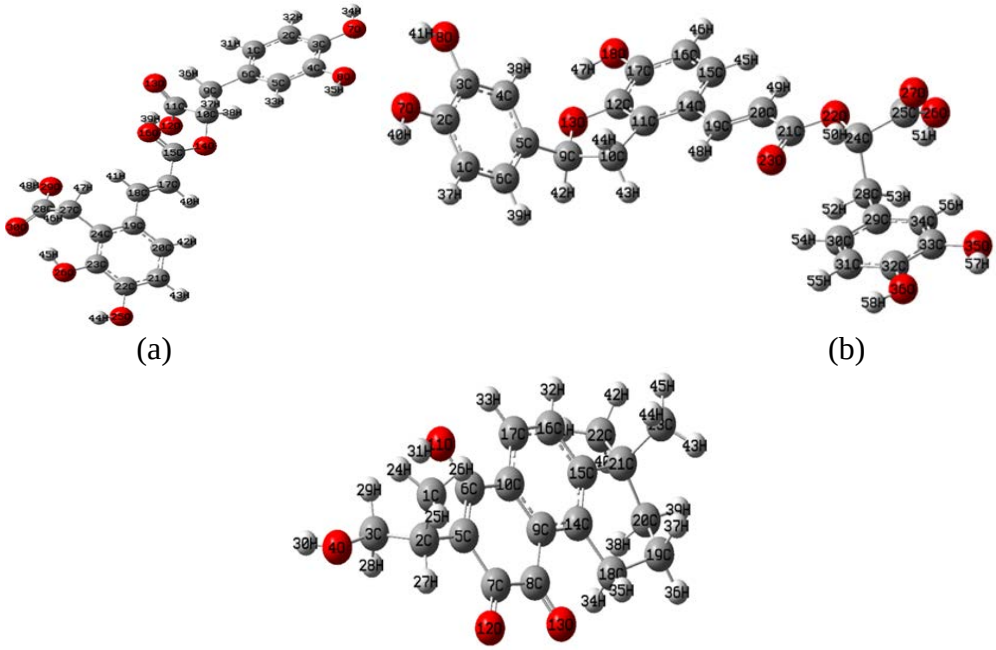


Figure 3. Conformation diagram of Neocryptotanshinone and target protein HMG-CoA reductase (1HWI)

3.3 Analysis of active sites

According to the molecular docking conformation map of Salvianolic Acid D, Salvianolic Acid C, Neocryptotanshinone and target protein, the target small molecules are numbered, and the results are as follows:



(a) (b)

(c)

Figure 4. (a) Molecular structure diagram of Salvianolic Acid D

(b) Molecular structure diagram of Salvianolic Acid C

(c) Molecular structure diagram of Neocryptotanshinone

The short contact distance between the three small molecule compounds and their adjacent amino acid residues is sorted out. The results are shown in the following table:

Table 3. Distance between target small molecule and protein crystal amino acid residues

Salvianolic Acid D…4F2A	Distance(Å)	Salvianolic Acid C…1LPB	Distance (Å)	Neocryptotanshinone…1HWI	Distance(Å)
O7…Ser230/H	1.90	O8…Ile248/HG23	4.00	O4…Ala751/O	2.91
O12…Ala195/HB1	2.82	O18…Asp247/OD1	3.00	O4….Lys735/N2	2.82
O13…Gln199/H	2.80	O18…Asp247/OD2	3.40	H32…Glu559/OE2	3.51
O14…Ser230/HG	4.00	O35…Arg337/HH21	2.00	H43…Glu559/O	2.60
O16…Ala202/HB2	4.41	O36…Arg337/HH22	1.90	O12…Asn755/ND2	2.71
O25…Ile215/HG23	2.61	H56…Lys367/H22	2.90		
O26…Cys13/HG	4.00				
O29…Lys13/HG	2.21				
O30…Leu261/HD22	4.42				

Through the above data, the binding mode and interaction force of the target small molecule compound with the target are analyzed. From the docking results of Salicylic Acid D and 4F2A in Figure 4(a), within the range of 3Å around the small molecule ligand, the ligand forms two hydrogen bonds with the amino acid residues Ser230 and Gln199 in the crystal structure. The dotted line represents the hydrogen bond), and the bond lengths are 1.90Å and 2.80Å, respectively. From the docking results of Salvianolic Acid C and 1LPB in Figure 4(b), the O at the 35th position of the small molecule and the O at the 36th position is respectively in the Arg337. HH22 in HH21 and Arg337 forms a total of 2 hydrogen bonds, and the distance between the corresponding hydrogen bond atoms is 2.00 Å and 1.90 Å, respectively. According to Fig. 4 (c), the schematic diagram of the docking results of Neocryptotanshinone and 1HWI shows that the interaction between the bulk molecule and the protein crystal mainly depends on the hydrogen bonding force. The ligand molecule forms two hydrogen bonds with Lys735 and Asn755 in the crystal structure, and the bond lengths are 2.82 Å and 2.71 Å, respectively.

4. Conclusion

In this study, computer simulation molecular docking technology was applied to the screening of medicinal substances of Shuang Dan capsule active ingredients, and the interaction mechanism between small molecule compounds and target proteins was explored based on atomic and molecular levels [14]. For the three targets that may cause hyperlipidemia, six potentially active small molecules were virtually screened from the existing literature reports and isolated and identified 157 target small molecule compounds, among which Salvianolic Acid D, Salvianolic Acid C, Neocryptotanshinone three kinds of candidate small molecules have a significant effect on the target protein; on this basis, further analyze the mode of action of the three lipid-lowering components and amino acid residues around the target crystal, and compare the interaction forces. Compared with traditional screening drug components, computer simulation can high-quality screening of potentially high-activity small molecules from complex natural Chinese medicine ingredients, avoiding the blindness of late in vitro activity experiments, and helping to deepen Salvia miltiorrhiza and Cortex moutan's application field, to increase the research and development of new drugs [15].

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