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Potential synergistic effects of Chinese herbal prescription FTZ components detected in blood towards hepatic lipid-modulating targets

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Hyperlipidemia;
Multi-target therapeutics;
Molecular docking;
Virtual screening;
Polypharmacology

Summary

Objective: Our goal in this study aims to explain the polypharmacological mechanism at the molecular level responsible for the effectiveness of a traditional Chinese medicine (TCM) prescription FTZ to treat hyperlipidemia and related disease.

Design: By MDL® ISIS_Base 2.5, we constructed a compound database based on the FTZ constituents, which were detected in the rat serum after oral administration of the TCM through ultra-performance liquid chromatography/quadruple-time-of-flight mass-spectrometry (UPLC/Q-TOF-MS/MS) method. After validation of the virtual docking system, we used molecular screening by LigandFit which is a computational method for the shape-directed rapid docking of ligands to target protein active sites, to investigate the interactions between the components in database and lipid-modulating targets in the liver.

Abbreviations: TCM, traditional Chinese medicine; UPLC/Q-TOF-MS, ultra-performance liquid chromatography/quadruple-time-of-flight mass-spectrometry; RC, Rhizoma Coptidis; RSM, Radix Salvia Miltiorrhiza; TC, total cholesterol; TG, total triglyceride; LDL-C, low density lipoprotein cholesterol; HDL, high density lipoprotein; HMGR, HMG-CoA reductase; SQS, squalene synthase; OSC, oxidosqualene cyclase; CETP, cholesteryl ester transfer protein; LXR, liver X receptor; FXR, farnesoid X receptor; PPAR, peroxisome proliferator-activated receptors; SAR, structure—activity relationship; MMFF, Merck Molecular Force Field.

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Results: In the prescription FTZ ingredients, there were sixteen constituents including jatrorrhizine, *etc*. showed potential effects towards the hyperlipidemia-related targets: HMG-CoA reductase (HMGR), squalene synthase (SQS), oxidosqualene cyclase (OSC), cholesteryl ester transfer protein (CETP), liver X receptor (LXR), farnesoid X receptor (FXR) and peroxisome proliferator-activated receptors ($PPAR_{\alpha}$ and $PPAR_{\gamma}$). Among the eight herbs in prescription FTZ, *Rhizoma Coptidis* (RC) plays the most important role in whole effect from FTZ on hyperlipidemia related disease.

Conclusions: Our research demonstrated that Chinese medicine formula FTZ has multi-target synergistic effect on hyperlipidemia and suggests the pharmacodynamic material basis could be jatrorrhizine, berberrubine, berberine and salidroside.

Introduction

Despite the obvious therapeutic advances over the past 50 years, cardiovascular disease is still one of the most important leading causes of death. Hyperlipidemia is one of the main risk factors for atherosclerosis, coronary heart disease and other cardiovascular diseases.[1,2] Controlling the lipid to a suitable level can offer substantial benefits for subject health as statins demonstrated.[3] However, due to rhabdomyolysis and hepatotoxicity induced by statins in some intolerable patients, alternative and complimentary therapies to treat hyperlipidemia are continually pursued.[4]

Fufang Zhenzhu Tiaozhi (FTZ) recipe, the patentable Chinese herbal prescription, is constituted with Rhizoma Coptidis (RC), Fructus Ligustri Lucidi, Herba Cirsii Jeponici, Radix Salvia Miltiorrhiza (RSM), Radix Notoginseng, Cortex Eucommiae, Fructus Citri Sarcodactylis and Radix Atractylodes Macrocephala. FTZ has been prescribed for 15 years to regulate abnormal lipid metabolism for treatment of hyperlipidemia, atherosclerosis, and related disease.[5,6] Clinical practice and *in vivo* experiments illuminated that FTZ can lower total cholesterol (TC), total triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) in blood while increasing high density lipoprotein (HDL),[7,8] but the accurate mechanism of these effects remains largely not understood.

In the information age, computational or *in silicon* methods including 2D or 3D molecular structure databases construction, SAR associated pharmacophore modelling, drug target homology modelling and molecular docking, are helping us to accelerate the entire process of drug research and development pipeline. Among these methods, the docking-based virtual screening which is to computationally simulate the molecular recognition process carries considerable weight in rational drug design. Molecular docking based on receptor theory originated from 19th century can be thought as similar interaction between lock and key. Scoring functions in molecular docking are commonly used to predict the strength of association or binding affinity between two molecules. The method by scouting structures from a large chemical library has been applied to TCM recently to discovery lead compounds among the complicated mixtures and interpret material basis of the specific effects.[9–11] It has the potential to speed up the rate of discovery at low lab cost by screening drug candidates from the clinical evidence-based TCM recipe.

According to serum pharmacology, active components in a mixture should be the ones which can be absorbed into the blood to reach the target organ. Compared to the previous TCM compound library construction based on the literature report,[9–11] we established a sensitive UPLC/Q-TOF-MS method for analysis of the constituents in rat serum after oral administration of FTZ, 27 prototype components of FTZ and 7 metabolites were identified *in vivo*.[12] The efficacy of FTZ for modulating lipid disorder could be originated from the 34 identified constituents in serum. Hereby we constructed chemical library based on absorbed constituents into blood by MDL® ISIS 2.5 to improve the hit and decrease the false positive rate.

In the present study, we use the docking-based virtual screening to gives a molecular description of the possible multi-target effect and a pharmacodynamic material basis of FTZ effect in clinic. The potential active components from virtual screening will help us to explore the synergistic mechanism about the lipid-regulating effect of FTZ and points the way to discover new chemical entities from the applied TCM recipe responsible for improving lipid disorder.

Methods

Construction of the compound ligand database from FTZ

Constituents in rat serum after oral administration of FTZ were detected and identified using UPLC/MS-MS, including alkaloids, ginsenosides, pentacyclic triterpenes, *etc*. and their metabolites. These constituents' backbone and detailed information were showed and labelled in S_Figure 1 and S_Table 1 as supplementary data. We constructed chemical library based on these absorbed constituents into blood by MDL® ISIS/Base 2.5, a flexible desktop database management system for storing, searching, and retrieving chemical structures and associated scientific data. These possible ligands as .db format file were converted into 3D structures and energy optimizations were performed using the Ligand Minimization module based on Merck Molecular Force Field (MMFF) before docking.

Liver lipid-regulating targets and preparation

Through therapeutic target database,[13] more than twenty proteins are regarded as the targets of hyperlipidemia,