Molecular Mechanism of Danxiong Tongmai Granules in Treatment of Coronary Heart Disease

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Abstract

Background: Network pharmacology has been widely adopted for mechanistic studies of Traditional Chinese Medicines (TCM). The present study uses network pharmacology to investigate the main ingredients, targets and pathways of Danxiong Tongmai Granules (DXTMG) in the treatment of coronary heart disease (CHD). We aim to validate our findings using molecular docking and molecular dynamics simulations.

Methods: TCM compounds and targets were identified via searches in the BATMAN-TCM database, and the GeneCards database were used to obtain the main target genes involved in CHD. We combined disease targets with the drug targets to identify common targets, and draw a Venn diagram to visualize the results. The "TCM-compound-target" network was plotted using Cytoscape 3.7.2 software and a protein-protein interaction (PPI) network was constructed using the STRING database from which core targets were obtained. Gene ontology (GO) function analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed for common drug-disease targets using R Version 4.0.4 (64 bit) software. Molecular docking of core protein-small molecule ligand interaction was modeled using AutoDock Vina software. A simulation of molecular dynamics was conducted for the optimal protein-ligand complex obtained by molecular docking using Amber18 software.

Results: 162 potential targets of DXTMG involved in CHD were identified. These included *INS*, *ALB*, *IL-6* and *TNF* according to PPI network studies. GO enrichment analysis identified a total of 3365 GO pathways, including 3049 biological process pathways (BP) concerned with the heart and circulatory system; 109 cellular component (CC) pathways, including cation channels and membrane rafts and 207 molecular function (MF) pathways related to receptor ligands and activators. KEGG analysis revealed a total of 137 pathways ($p < 0.05$), including those related to AGE-RAGE signaling associated with diabetic complications, fluid shear stress and atherosclerosis. Molecular docking revealed the highest binding energy for Neocryptotanshinone II (the key compound of DXTMG) and *TNF*. Molecular dynamics simulation indicated stable binding for *TNF*-Neocryptotanshinone II with strong hydrophobic interactions mediated predominantly by the hydrophobic residues, Leu279, Val280 and Phe278 plus hydrogen-bonding with Leu279.

Conclusion: The present study reveals novel insights into the mechanism of DXTMG in treating CHD. DXTMG can influence oxidative stress–inflammation response and regulating cardiomyocytes, thereby reducing the occurrence and development of CHD.

1. Introduction

Coronary heart disease (CHD) occurs when atherosclerosis causes luminal stenosis or occlusion, leading to insufficient blood supply to the coronary artery and failure to meet the demands of myocardial metabolism^[1]. According to the American Heart Association, 15.5 million adults in the United States suffered from CHD in 2016^[2]. CHD has a serious impact on the patient's quality of life and is

characterized by poor prognosis causing high morbidity and mortality rates. Therapeutic approaches commonly taken by Western medicine have many side effects, especially during long-term administration. For example, a 4 month course of statins may contribute to liver damage with raised ALT and AST levels and development of hepatocyte necrosis, liver fibrosis and increased inflammatory responses^[3]. Moreover, there is an increased risk of new-onset diabetes^[4]. By contrast, Traditional Chinese Medicine (TCM) often has multi-target and multi-pathway synergistic effects and low incidence of toxic side effects. Thus, TCM has great potential for the treatment of CHD.

Danxiong Tongmai Granules (DXTMG) have become increasingly popular for the treatment of CHD. The clinical investigations of Yao *et al.* demonstrated good efficacy for DXTMG in patients with mild, moderate and severe CHD without toxic side effects^[5]. Moreover, Tang found improvements in arterial elasticity and hypertension after treatment of patients with cerebrovascular disease^[6]. In addition, Shi found decreases in platelet activation, as assessed by flow cytometry, after administration of DXTMG^[7]. To date, studies on DXTMG have been predominantly focused on clinical observation and little progress has been made in explaining its molecular mechanisms.

Network pharmacology is a novel pharmaceutical research method for the systematic analysis of "multi-component-multi-target-multi-pathway" characteristics and has been applied to TCM^[8, 9]. Molecular docking is an approach whereby small ligand molecules are modeled within the receptor binding site so that spatial interactions may be explored. Using flexible and semi-flexible docking, receptor-ligand interactive forces may be assessed enabling prediction of binding mode and affinity^[10]. Molecular dynamics allows simulation of various ligand and receptor movements via Newtonian mechanics to assess stability and flexibility. During the present study, core target receptors and ligands were identified through network pharmacology and interaction sites and binding characteristics analyzed using molecular docking and molecular dynamics simulations, so as to explore the mechanism of DXTMG in the treatment of CHD. The Flowchart is shown in Fig. 1.

2. Methods

2.1 Chemical compounds and target genes

The components of DXTMG include Radix Paeoniae Rubra, Ligusticum wallichii, Salvia miltiorrhiza, Polygonum multiflorum, Rhizoma corydalis, Cyperus rotundus, Carthamus tinctorius and Lycium barbarum. Key compounds with target proteins were retrieved from the BATMAN-TCM database (<http://bionet.ncpsb.org/batman-tcm/index.php>) under the condition of score cutoff ≥ 20 and $p < 0.05$ ^[11].

2.2 Targets of CHD

A total of 450 CHD target genes were obtained from the Genecards database (<https://www.genecards.org/>) by using "coronary heart disease" as the keyword and the criterion of Score

> 30^[12]. Target genes common to both DXTMG and CHD identified by the Venny online tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) and a Venn diagram constructed.

2.3 Construction of "TCM-compound-target" network

The effective compounds and drug targets were imported into Cytoscape 3.7.2 software and a "TCM-compound-target" network diagram was plotted^[13]. The built-in tool, Network Analyzer, was used to analyze the associations.

2.4 Construction of "protein-protein" interaction (PPI) network

Common target proteins were imported into the STRING database(<https://www.string-db.org/>)^[14] to construct a PPI network. The species was set to "Homo sapiens", the confidence > 0.4 and unconnected nodes were hidden. The PPI network diagram was plotted using Cytoscape 3.7.2 software. Core targets with the top 30 degree scores were plotted into a PPI histogram using R Version 4.0.4 (64 bit) software.

2.5 Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

GO and KEGG analyses were conducted using R package of clusterProfiler^[15].GO enrichment analysis covered biological process (BP), molecular function (MF) and cellular component (CC). The top 10 GO and the top 20 KEGG pathways were identified ($p < 0.05$).

2.6 Molecular docking

The five compounds with the highest scores in the "TCM-compound-target" network diagram and the four core proteins with the highest scores in the PPI network were subjected to molecular docking analysis using AutoDock Vina software^[16]. Binding energies were calculated. Compound structures were obtained from the PubChem database and the protein structures from the PDB database (<http://www.rcsb.org/>). Results were visualized by Pymol software. Binding energies were classified as follows: >5 : initial binding; >7 : stronger binding and >9: very strong binding^[17].

2.7 Molecular dynamics simulation

The amber18 software package was used to perform molecular dynamics simulation on the protein(target) and small molecule ligand (compound) obtained by molecular docking. ff14SB and general Amber force field (GAFF) parameters were used for proteins and small molecule ligands, respectively, and the AM1-BCC atomic charge was calculated using the ANTECHAMBER module. The protein-ligand complex was loaded into the leap module and hydrogen atoms and antagonist ions were automatically added to neutralize the charge. The TIP3P explicit water model was selected and periodic boundary conditions were set. The workflow of molecular dynamics simulation included energy minimization, heating, equilibration and production dynamics. Heavy atoms of proteins (small molecules) were constrained and water molecules subjected to 10,000 steps of energy minimization

(including 5000-step steepest descent method and 5000-step conjugate gradient method). The system was slowly heated to 300 K within 50 ps and equilibrated for 50 ps under the NPT ensemble. Molecular dynamics simulation was conducted on the system under the NPT ensemble for 50 ns (25,000 steps in total) with a time step of 2 fs. The trajectory data were saved every 10 ps, followed by analysis using the CPPTRAJ module. The free energy of binding for ligands and proteins was calculated using the MMPBA.py module.

3. Results

3.1 Screening of active pharmaceutical ingredients and targets

Compounds from DXTMG retrieved from the BATMAN-TCM database included 9 compounds from *Radix Paeoniae Rubra*, 91 from *Ligusticum wallichii*, 39 from *Salvia miltiorrhiza*, 16 from *Polygonum multiflorum*, 37 from *Rhizoma corydalis*, 12 from *Cyperus rotundus*, 22 from *Carthamus tinctorius* and 1 from *Lycium barbarum*. Seven replicates were removed to leave 220 compounds in total (Supplementary Information Table S1). In addition, a total of 11,121 drug targets were retrieved, including 127 targets of *Radix Paeoniae Rubra*, 2163 of *Salvia miltiorrhiza*, 4970 of *Ligusticum wallichii*, 300 of *Polygonum multiflorum*, 1533 of *Rhizoma corydalis*, 1074 of *Cyperus rotundus*, 913 of *Carthamus tinctorius* and 41 of *Lycium barbarum*. Removal of replicates left a total of 1506 targets.

3.2 Screening of CHD target genes and construction of "TCM-Disease" intersect targets

A total of 6810 CHD targets were obtained from the Genecards database. After those with a relevance score > 30 had been screened out, a total of 450 targets remained. A total of 162 targets common to DXTMG and CHD were identified using the Venny online tool (Fig. 2).

The blue part represents the targets of DXTMG, the red part represents the genes associated with CHD, and the intersection represents the potential targets for the intersect of DXTMG decoction in the treatment of CHD.

3.3 Construction of "TCM-compound-target" network

The data were visualized by Cytoscape 3.7.2 software. A "TCM-compound-target" network (Fig. 3) was constructed, and its nodes were analyzed. The compounds with the top 10 Degrees were Tanshinone II, Neotanshinone C, Neocryptotanshinone I, Miltionone I, Patchoulone, Dihydrokaranone, Rotundone, Miltirone, Dehydromiltirone, Yuehchukene. These compounds are likely to be the major chemical components of DXTMG with efficacy in the treatment of CHD. The core compounds are shown in Supplementary Information Table S2.

Green ellipse: TCM; blue triangle: compound; purple rectangle: target

3.4 Construction of PPI network

The 162 CHD target proteins of DXTMG were imported into the STRING database and resulting data were imported into Cytoscape 3.7.2 software. A PPI network diagram was constructed (Fig. 4). A PPI histogram for targets with the top 30 Degrees was plotted using R Version 4.0.4 (64 bit) software (Fig. 5). Core targets with the top 4 Degrees were INS, Alb, IL-6 and TNF.

(X-axis: Degree; Y-axis: genes)

3.5 GO enrichment and KEGG pathway enrichment analysis

The results of GO enrichment analysis showed that there were 3365 GO pathways in total, including 3049 pathways of BP (heart and circulatory system), 109 pathways of CC (cation channels and membrane rafts) and 207 pathways of MF (receptor ligand and activator). The relationships between BP and targets are shown in Fig. 6 and the GO analysis results of BP, CC and MF are shown in Fig. 7.

A total of 137 KEGG pathways were obtained. DXTMG mainly regulated the Lipid and atherosclerosis, MAPK signaling pathway, Fluid shear stress and atherosclerosis, Diabetic cardiomyopathy. The top 20 KEGG pathways are shown in Fig. 8.

3.6 Molecular docking

Molecular docking was investigated for core compounds: Miltionone I, Neocryptotanshinone li, Neotanshinone C, Patchoulone and Tanshinone II and core targets: ALB, IL-6, INS and TNF. Binding energies were <-7 kcal/mol, indicating strong binding capacity between compounds and targets. The strength of binding between Neocryptotanshinone li and TNF was the highest (-14.09 kcal/mol). This finding suggests that this is a major compound-target, interaction. The molecular docking results of compounds and target proteins are shown in Supplementary Information Table S3 and the molecular docking diagram is shown in Fig. 9.

Neocryptotanshinone li interacts with residue SER-159 of TNF.

3.7 Results of molecular dynamics simulation

Based on binding energy results, molecular dynamics simulation was performed for *TNF*-Neocryptotanshinone li. The root-mean-square deviation (RMSD), radius of gyration (Rog), root-mean-square fluctuation (RMSF) curve and binding free energy were calculated.

The RMSD curve represented variations in protein conformation. There were certain fluctuations in early stages but stability was established after 20 ns. The results show that ligand-protein interactions do not cause great changes in protein conformation and binding is relatively stable.

The Rog curve displayed the compactness of the overall protein structure. It was found that the Rog remained stable throughout the simulation process, consistent with the RMSD result, indicating a stable

and compactly folded protein conformation. Hence, ligand binding does not affect protein stability.

The RMSF curve displays variations in the conformation of amino acid residues. Residue flexibility was higher in the middle region of the protein. The middle region was dominated by loop-based polypeptide chains, giving greater flexibility. The results of RMSD, Rog and RMSF are shown in Fig. 10.

The trajectory of the RMSD plateau (30–50 ns) was calculated, allowing calculation of free energy of binding for ligand-protein interactions based on the MMGBSA equation. The free energy of binding was –11.7 kcal/mol, suggesting general binding capacity. Van der Waals potential (-20 kcal/mol) and electrostatic interaction (-29 kcal/mol) were conducive to binding while polar solvation (EGB, 41 kcal/mol) opposed binding. Free energies of binding are shown in Supplementary Information Table S4.

The *TNF*-Neocryptotanshinone li binding sites had strong hydrophobicity and hydrophobic residues, such as Leu279, Val280 and Phe278, were involved. In addition, the ligand may participate in a hydrogen-bond interaction with Leu279. The *TNF*-Neocryptotanshinone li interaction diagram is shown in Fig. 11.

(A) RMSD diagram ; (B) Rog diagram ; (C) RMSF diagram.

(A) Schematic diagram of binding sites; (B) 3D diagram of ligand-receptor interaction; (C) 2D diagram of ligand-receptor interaction.

4. Discussion

The current study presents evidence that Tanshinone II, Neotanshinone C, Neocryptotanshinone li, Miltionone and Patchoulone are the major compounds of DXTMG with potential pharmacological activity. Previous work at the cellular level has established that Tanshinone II_A greatly relieves myocardial inflammatory infiltration and oxidative damage caused by myocardial ischemia-reperfusion injury in rats and reduces leakage of lactate dehydrogenase and production of malondialdehyde, thereby protecting against myocardial ischemia-reperfusion injury^[18]. The findings of Weng *et al.*^[19] demonstrated that Tanshinone II_A has an estrogen-like effect resulting in protein kinase B (*Akt*) activation and suppression of cardiomyocyte apoptosis on activation by insulin-like growth factor II (*IGF-II*). This suggests a role for Tanshinone II_A as a potential selective estrogen receptor modulator in the prevention of myocardial apoptosis and treatment of cardiovascular diseases. Moreover, Li *et al.*^[20] demonstrated a role for Tanshinone II_A in suppressing mitogen-activated protein kinase (*MAPK*) and down-regulating c-fos expression, thus reducing intimal hyperplasia and vascular smooth muscle cell proliferation in rats with carotid artery balloon injury. In addition, Wu *et al.*^[21] showed that Neocryptotanshinone suppresses lipopolysaccharide-induced inflammatory factors, including *TNF- α* and *IL-6*, in rats. Neocryptotanshinone also reduces lipopolysaccharide-induced production of nitric oxide. The pharmacological and computer-based investigations of Maione *et al.*^[22] demonstrated a concentration-dependent activity of Neocryptotanshinone in inhibiting platelet aggregation and antagonizing G protein-coupled P2Y₁₂ platelet ADP receptor.

The PPI analysis of the current study identified *INS*, *ALB*, *IL-6* and *TNF* as the major targets of DXTMG in CHD treatment. Accumulation of inflammatory cells around damaged vascular tissues may promote plaque formation through the secretion of inflammatory mediators. Therefore, inflammatory markers are considered risk factors for CHD^[23, 24]. Insulin resistance (*IR*), a predisposing factor for cardiovascular diseases such as CHD^[25], is characterized by increased levels of inflammatory cytokines and expression of adhesion molecules, vascular dysregulation and vascular endothelial injury, ultimately resulting in atherosclerosis^[26]. *INS* enhances glucose uptake and utilization, thereby protecting the ischemic-hypoxic myocardium. Blood glucose elevation is a risk factor for cardiovascular diseases and the hypoglycemic effect of *INS* is central to blood glucose regulation^[27]. High-levels of *INS* increase angiotensin II, through the renin-angiotensin system, and promote production of reactive oxygen species, thereby facilitating inflammatory and oxidative stress responses^[28]. Normal concentrations of albumin inhibit platelet activation and aggregation and apoptosis of vascular endothelial cells, thereby reducing CHD risk^[29]. Moreover, activation of the Apelin/G protein-coupled receptor (*APJ*) causes blood vessel dilation, reducing blood pressure and increasing myocardial contractility and relaxation. Apelin inhibits the potential activity of *AVP* neurons and *AVP* release, reducing plasma osmotic pressure and *ALB* concentration and increasing CHD risk. Therefore, maintenance of stable plasma *ALB* concentrations can help reduce the occurrence of CHD^[30, 31]. Scheller J *et al.*^[32] showed a positive correlation between *IL-6* levels and incidence of severe coronary artery lesions. *IL-6* promotes macrophage activation and formation of foam cells, stimulating expression of macrophage low-density lipoprotein receptors and triggering matrix metalloproteinases to destabilize atheromatous plaques, resulting in plaque rupture^[33]. It is known that vascular endothelial injury stimulates inflammatory receptors and release of inflammatory factors, such as *TNF-α* and *IL-6*, followed by thrombosis and plaque rupture^[34]. The suppression of smooth muscle cell proliferation, an effect that correlates with down-regulated expression of *TNF-α* and *IL-6*, may improve the stability of atherosclerotic plaques^[35]. *TNF-α* stimulates apoptosis through increasing protease activity which results in increased basal metabolism^[36].

The current study used KEGG pathway enrichment analysis to demonstrate that fluid shear stress and atherosclerosis, the hypoxia-inducible factor-1 (HIF-1) signaling pathway, adrenergic signaling in cardiomyocytes and the AGE-RAGE signaling pathway in diabetic complications were implicated in the action of DXTMG in CHD treatment. Atherosclerosis is involved in the pathological development of CHD when plaque formation-induced changes in shear stress affect the normal function and phenotype of vascular wall endothelial cells^[37]. It has been established that low shear stress up-regulates expression of intercellular adhesion molecule-1 on the endothelial cell surface, promoting activation of endothelial cells^[38]. A further consequence is phosphorylation of platelet endothelial cell adhesion molecule-1, activating the MAPK pathway and worsening inflammation to promote CHD. HIF-1, a hypoxia-induced DNA-binding protein, induces increased expression of HIF-1α during myocardial ischemia and hypoxia, thereby protecting the myocardium^[39]. Moreover, HIF-1 induces endothelial cell dysfunction, proliferation, angiogenesis and inflammation, thus playing a role in the pathogenesis of atherosclerosis via various pathways^[40]. Via its action on the α1 receptor in vascular smooth muscle, epinephrine promotes

vasoconstriction and increases aortic diastolic pressure, thereby raising coronary perfusion pressure^[41]. Adrenergic signaling in cardiomyocytes enhances myocardial contractility, resulting in vascular dilation of both heart and liver and vascular contraction of both skin and mucosa, thereby exerting a therapeutic effect on CHD. Activated AGE/RAGE signaling which enhances inflammation, oxidative stress and vascular smooth muscle cell apoptosis, thus promoting the development of atherosclerosis, plays a significant role in CHD through the above mechanisms^[42].

We present results of molecular docking experiments which revealed stable binding between components of DXTMG and receptor proteins with binding energy of the *TNF*-Neocryptotanshinone li interaction being the highest (-14.09 kcal/mol). Moreover, the molecular dynamics simulations of the current study show stable *TNF*-Neocryptotanshinone li binding. Binding sites had strong hydrophobicity and hydrophobic residues, such as Leu279, Val280 and Phe278, were involved. In addition, the ligand may undergo a hydrogen-bond interaction with Leu279.

5. Conclusion

The current study constructed a "TCM-compound-target" network and PPI network to investigate the role of DXTMG in the treatment of CHD using the approach of network pharmacology. Findings were validated by molecular docking and molecular dynamics simulation. Our findings reveal DXTMG can influence oxidative stress–inflammation response and regulating cardiomyocytes, thereby reducing the occurrence and development of CHD and expose avenues for further investigation through animal and clinical experiments.

Abbreviations

TCM: Traditional Chinese medicines; DXTMG: Danxiong Tongmai Granules; CHD: Coronary Heart disease; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: Protein-protein interaction; INS: Insulin; ALB: Albumin; IL6: Interleukin 6; TNF: Tumor necrosis factor; BP: Biological processes; CC: Cellular component; MF: Molecular function; BATMAN-TCM: Bioinformatics Analysis Tool of Molecular mechanism of Traditional Chinese Medicine; GAFF: General Amber Force Field; RMSF: Root mean square fluctuation; RMSD: Root mean square deviation; IGF-II: Insulin-like growth factor II; Akt: Protein kinase BMAPK: Mitogen-activated protein kinase; IR: Insulin resistance; HIF-1: Hypoxia-inducible factor-1; PDB: Protein Data Bank.

Declarations

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Authors' Contributions

The design of the study was performed by ZXH, JHY collected the data. RPY prepared Figs. The manuscript written by JHY and RPY. LL and SJZ and RXJ reviewed and edited the manuscript. JHY and RPY contributed equally to this work. All authors have read and approved the manuscript.

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Data Availability

All data generated or analyzed during this study are available from public databases, published articles and supplementary materials.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Competing interests

The authors declare no conflicts of interest.

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Figures

Figure 1

Flowchart of investigating the mechanism of DXTMG in CHD treatment

Figure 2

Venn diagram of DXTMG and CHD targets.

Figure 4

PPI network of targets of DXTMG and CHD. As the Degree value increases, the circle changes from red to blue, from small to large. As the Combined score increases, the connection line changes from red to blue, from thin to thick.

Figure 5

Core targets with the top 30 Degrees (X-axis: Degree; Y-axis: genes)

Figure 6

Relationship between BP and targets. Lines with different colors represent the relationships between different BPs (given in key to diagram) and genes with the size of the circle being proportional to the number of enriched genes.

Figure 7

GO analysis results.X-axis: number of significantly different genes; Y-axis; GO term.

Figure 8

Results of KEGG pathway enrichment analysis.X-axis: number of significantly different genes; Y-axis: KEGG term.

Figure 9

Molecular docking of TNF and Neocryptotanshinone II. Neocryptotanshinone II interacts with residue SER-159 of TNF.

Figure 10

Molecular dynamics simulation results for TNF-Neocryptotanshinone li (A) RMSD diagram ; (B) Rog diagram ; (C) RMSF diagram.

Figure 11

Residues involved in interactions in the TNF-Neocryptotanshinone li . (A) Schematic diagram of binding sites; (B) 3D diagram of ligand-receptor interaction; (C) 2D diagram of ligand-receptor interaction.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1S4.rar](#)