**Construction of Pharmacological Network and Molecular Docking
Analysis of Key Molecular Components of Jianpi Yiqi Formula for
Idiopathic Thrombocytopenic Purpura**

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Abstract

Purpose: To perform network pharmacological analysis so as to identify and screen the active ingredients of Jianpi Yiqi Formula; find its core target and explore its mechanism in the treatment of idiopathic thrombocytopenic purpura (ITP).

Materials and Methods: A network pharmacology approach was used to inquire and screen the active ingredients from the Traditional Chinese Medicine System Pharmacology (TCMSP) database for potential active compounds that are commonly contained in the Jianpi Yiqi formula. The Swiss Target Prediction database was used for the prediction of the active ingredient's target of the action; the genecard database was used to search for target genes associated with ITP and to screen for target genes, in which the drug target was intersected with the disease target. Protein interaction network was constructed using string database software for GO and KEGG analysis to construct the "component/target/pathway" pharmacology network of Jianpi Yiqi granules therapy for ITP. Cytoscape 3.7.2 software was harnessed to visualize and integrate this network. The Virtua Drug web-based pharmacology website (<https://www.dockingserver.com>) was used to validate the regulatory relationship between key active compounds and critical pathway molecular signals by molecular docking.

Results: Two key active ingredients, quercetin, and kaempferol, were selected from hundreds of herbal ingredients referenced in online pharmacological studies. Molecular docking analysis revealed that quercetin and kaempferol could stably bind PI3K/AKT and exert inhibitory effects, respectively. It was also speculated that PI3K/AKT/mTOR pathway might be the critical pathway for the pharmacokinetic mechanism of Jianpi Yiqi Granules.

Conclusion: The present study suggests the multi-component effect characteristic of the treatment of ITP with Jianpi Yiqi granules, thus providing a theoretical basis for the clinical use of Jianpi Yiqi formula.

Keywords: ITP; Jianpi Yiqi Formula; Network Pharmacology

1. Introduction

Traditional Chinese Medicine (TCM), which is one of the greatest treasures of Chinese culture, has a long history in East and Southeast Asia. Through continuous development and innovation, removal of impurities, and retention of the essence, TCM has become one of the major forms of alternative medicine in East Asia, North America, and Europe.

Idiopathic thrombocytopenic purpura (ITP) is a common bleeding disorder that can lead to life-threatening petechiae, ecchymosis, internal bleeding, and even cerebral and mucosal hemorrhage. The annual incidence rate of ITP in adults, which is most often found in women under 40 years of age, is 2-4/100,000. The mortality rate is almost 1% ^[1]. Numerous clinical and experimental studies have suggested that the Jianpi Yiqi formula (strengthening the spleen and benefiting the vital energy) has good efficacy in improving clinical bleeding symptoms and regulating immunity. Jianpi Yiqi formula, based on the formula for Jianpi with the addition or subtraction of herbal flavors, is composed of *Codonopsis pilosula* (dangsen), *Glycyrrhiza glabra* (gancao), *Wolfiporia extensa* (fu ling), *Astragalus* (huangqi), *Atractylodes macrocephala* (baizhu), and *Rubia cordifolia* (qiancao), and can be used in ITP treatment. At present, studies on the pathways and targets of action of this compound have not yet been reported in the literature. *Codonopsis pilosula*, *Glycyrrhiza glabra*, *Wolfiporia extensa*, *Astragalus*, *Atractylodes macrocephala*, and *Rubia cordifolia* are all Qi (vital energy) strengthening tonics. Therefore, the common components of the above herbs may have an essential role in ITP treatment.^[1]

Network pharmacology, an emerging discipline that has appeared over recent years, integrates systems biology, multidirectional pharmacology, computational biology, and network analysis, as well as other multidisciplinary techniques and content to explore disease and drug interaction mechanisms. Network pharmacology falls in line with the therapeutic concept of complex diseases. It has been widely used for the prediction of potential active ingredients, action targets in traditional Chinese medicine, and elaboration of the mechanisms of action ^[2]. In this study, we investigated the therapeutic mechanism of ITP through network pharmacology analysis and molecular docking studies of the key common components in Jianpi Yiqi

formula to provide a theoretical basis for its clinical use.

[2]

2. Materials and Methods

2.1 Database and analysis software

Network pharmacology research was primarily bioinformatics-based and was performed using network pharmacology databases and related software, including Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TCMSP), the String database (<https://string-db.org/>), the UniProt database (<https://www.uniprot.org/>), the genecards database (www.genecards.org), the Swiss Target Prediction database (<http://www.swisstargetprediction.ch/>), the Protein Data Bank database (PDB, <https://www.rcsb.org/>), PubChem database (<https://pubchem.ncbi.nlm.nih.gov>), the Virtua Drug web pharmacology site (<https://www.dockingserver.com>, which is an online molecular docking program), and Cytoscape 3.7.2.

2.2 Screening of key active ingredients in Jianpi Yiqi formula

The TCMSP database was used to retrieve each single herb from Jianpi Yiqi formula, and construct an ingredient database. By considering the recommended screening criteria in the TCMSP and the associated absorption, metabolism, distribution, and excretion (ADME/T) values of the drug in the body, the search was performed to identify the potentially active compounds contained in common components of Jianpi Yiqi formula, referring to the criteria of similarity ≥ 0.18 (drug-likeness (DL) ≥ 0.18) and oral bioavailability $\geq 40\%$ (OB $\geq 40\%$). The molecular structures of active compounds were confirmed using the PubChem chemical structure database platform, and the structure maps obtained from the database were stored in both MOL and SDF formats for further study.

2.3 Prediction of active ingredient target genes

The Swiss Target Prediction database predicted the selected active ingredients. First, we logged into the PubChem server to find the information of the main active ingredients of Jianpi Yiqi formula. We then uploaded the Canonical SMILES of the main active ingredients in the Swiss Target Prediction database to obtain the target of each active ingredient in combination with the 2D and 3D similarity metrics of known

ligands. The target information of the key active ingredients in the Jianpi Yiqi formula was obtained by combining the target information related to all active ingredients and removing duplicate targets. The protein database IDs (PDB IDs) of the filtered protein targets were imported into the UniProt database, and the predicted targets of the active ingredients of the Jianpi Yiqi formula were obtained by searching and transformation. The retrieved targets were transformed by the UniProt database and corrected to the official abbreviation of the target.

2.4 Screening of core targets and construction of protein interaction networks

The Genecards database was searched to find ITP-associated disease targets using ITP as a keyword, and the protein database identifiers (PDB IDs) of the filtered disease targets were imported into the UniProt database. Then, the disease targets of ITP were obtained by searching and transformation. The retrieved targets were transformed by the UniProt database and corrected to the official abbreviation of the target. The intersection of the disease target and the component target was taken to obtain the core target.

2.5 Target pathway annotation analysis

On the STRING gene annotation platform, the corresponding species, *Homo sapiens* "*H.sapiens*" was selected, and the resulting Core targets were imported into the STRING database. The threshold was set to $P < 0.01$ for Gene Ontology (GO) Annotation and KEGG signaling pathway analysis.

2.6 Molecular docking and binding affinity

Molecular docking is often used to study the interactions between proteins and active small molecules (i.e., the ligand-receptor relationship between drug molecules and proteins). The crystal structures of proteins were obtained from the PDB database, while the pharmacological molecular structures were obtained from the PubChem database. Active compounds (MOL2 format) and target protein PDB IDs were uploaded to Virtua Drug Network Pharmacology website (<https://www.dockingserver.com>). The smaller the binding free energy between molecules and targets was, the more stable the ligand-receptor was. We investigated

the binding of key components to PI3K/AKT/mTOR molecules, a key regulatory pathway for platelet autophagy/apoptosis, using molecular docking techniques, and Virtua Drug online database.

2.7 Construction of compound-target-pathway networks

Compound-target and target-pathway networks were constructed to systematically understand the complex relationships between compounds by using Cytoscape Version 3.7.2 (<https://cytoscape.org/>).

3. Results

3.1 Network-based pharmacological study of Jianpi Yiqi formula for ITP treatment

A total of 106 ingredients with well-defined pharmacological activities in the Jianpi Yiqi formula were screened for drug similarity (DL) ≥ 0.18 and oral bioavailability (OB) $\geq 30\%$ through the TCMSP database, so as to clarify the mechanism of ITP treatment and molecular targets. Among these ingredients, *Glycyrrhiza glabra* contained 69 active components; Astragalus contained 14 active components; *Codonopsis pilosula* 12 active components; Rubia cordifolia 11 active components; *Atractylodes macrocephala* 4 active components; *Wolfiporia extensa* contained 3 active components. As shown in the Wayne diagram of effective components in each component of the Jianpi Yiqi formula in **Figure 1-A**, the same pharmacologically active molecules are partially found in the components, with six common components being present in *Glycyrrhiza glabra*/Astragalus (MOL000422, MOL000354, MOL000098, MOL000417, MOL000239, and MOL000392), and MOL003896 being commonly found in *Glycyrrhiza glabra* and *Codonopsis pilosula*. These drug-active molecules may be the key pharmacological components.

Target prediction of the above active ingredients by the Swiss Target Prediction database yielded a total of 263 target genes. Five hundred fifty-five relevant target genes of ITP were obtained through Genecard and other databases, and 33 core targets with intersections were selected to construct PPI networks and perform KEGG analysis. KEGG is a functional enrichment, i.e., the gene set (multiple genes) that may be concentrated on specific functions. As shown in **Figure 1-f**, we discovered that the mechanism of the effect of the Jianpi Yiqi formula on ITP might involve the

PI3K/AKT signaling pathway.

GO analysis is a functional annotation that includes components, bioprocess, and functional analyses. It shows pathway terms or GO terms in which each gene may be involved in, with no thresholds. GO analysis gives a rough idea of biological functions, pathways, or cellular localizations the differential genes are enriched in. The most attention is usually paid on the results of the functional analysis and annotation of the bioprocess, which can be seen in **Figure 1-i**. The aforementioned pharmacological components mainly function in a protease-binding manner and act through the bioprocess that promotes cell proliferation and negatively regulates apoptosis and programmed cell death. PI3K/AKT signaling resulted as a key regulatory pathway for platelet autophagy/apoptosis, and the combination of KEGG analysis. In addition, GO analysis suggested that the key active molecules screened from the Jianpi Yiqi formula may treat ITP by participating in the PI3K/AKT signaling pathway that regulates platelet apoptosis.

Figure 1 Network pharmacological analysis of the treatment of ITP with Jianpi Yiqi formula. (a) Wayne's diagram of important active molecules of each component of Jianpi Yiqi formula. (b) Predicted target network of active compounds of Jianpi Yiqi formula. (c) Wayne's diagram of drug targets of Jianpi Yiqi formula and ITP-associated target genes. (d) Compound-Jianpi Yiqi formula/ITP putative therapeutic target network map. (e) PPI network of key target genes of Jianpi Yiqi formula. (f) KEGG analysis of key target genes; (g-i) GO analysis of key target genes.

3.2 Molecular docking studies

To further clarify the effect of the key active molecules MOL000422, MOL000354, MOL000098, MOL000417, MOL000239, MOL000392 and MOL003896 on the PI3K/AKT signaling pathway and seek key targets for regulation, the online database was leveraged (<https://www.dockingserver.com>), and molecular docking techniques were used to study the modalities through which the above key active molecules were binding the PI3K/AKT/mTOR molecules on autophagy/apoptosis key regulatory pathway and the key target proteins Beclin1/BCL-2/BCL-XL/BAX/CASP3 molecules on autophagy/apoptosis pathway. The analysis revealed that quercetin had highly desirable molecular docking results with the PI3K protein (the total intermolecular free energy of the molecular binding is -5.15 kcal/mol), and the binding frequency of 100%. The binding surface was 661.932 (**Figure 2**). Kaempferol had a very desirable molecular docking result with the AKT1 protein (the total intermolecular free energy for molecular binding is -4.99 kcal/mol; its binding frequency was 100% and the molecular binding surface was 449.406; **Figure 3**). These indicated that quercetin, kaempferol, and their respective target molecule PI3K/AKT had low binding energy and large binding surface, which allowed for the formation of stable complexes that inhibit the target molecules. The results suggest that quercetin and kaempferol can jointly inhibit the PI3K/AKT/mTOR signaling pathway and thus have an effect on cell autophagy/apoptosis.

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228 **Figure 2** Analysis of the molecular docking results of kaempferol and AKT1 protein. (a)
 229 3D simulation schematic; (b) detail simulation diagram; (c) 2D plot; (d) molecular binding
 230 energy; (e) position information of the bound molecules; (f) HB plot; (g) molecular docking
 231 analysis results.

Figure 3 Analysis of molecular docking results of quercetin and PI3K protein. (a) 3D simulation schematic; (b) detail simulation diagram; (c) 2D plot; (d) molecular binding energy; (e) position information of the bound molecules; (f) HB plot; (g) molecular docking analysis results.

4. Discussion

Patients with ITP have clinical symptoms such as physical lassitude, lack of energy, lazy speech, loss of appetite, fatigue, and chronic bleeding. TCM postulates that "weak temperament and failure of Qi to control blood" is the basic cause of ITP, which is also at the basis of the TCM theory in the treatment of chronic ITP with the Jianpi Yiqi formula [3]. The Jianpi Yiqi formula aims to strengthen the spleen with the addition and subtraction of herbal flavors, including *Atractylodes macrocephala*, *Codonopsis pilosula*, *Glycyrrhiza glabra*, *Wolfiporia extensa*, *Astragalus*, *Rubia cordifolia*, and donkey-hide gelatin. The Jianpi Yiqi formula can strengthen the spleen, tonify Qi, nourish the blood, and promote blood circulation. It also has significant efficacy in the treatment of ITP in clinical practice [3-6]. Yet, the pharmacokinetic

molecule and target of action of this compound still remain unclear, which limits its wide application.^[1]

Network pharmacological analysis revealed that quercetin and kaempferol might be the key pharmacokinetic molecules in the treatment of ITP by the Jianpi Yiqi formula. Previous studies have shown that quercetin [7] and kaempferol [8], which are both flavonoids, inhibit platelet activation, and prevent platelet phosphatidylserine (PS) exposure, thereby inhibiting platelet apoptosis. However, the specific mechanism through which they regulate platelet apoptosis remains unknown. Molecular docking studies have shown that quercetin and PI3K, and kaempferol and AKT are complete stable complexes that may be involved in the co-suppression of the PI3K/AKT/mTOR signaling pathway. The PI3K/AKT/mTOR axis is a critical signaling pathway that regulates platelet activation, apoptosis, and autophagy. In 2018, Dai Kesheng *et al* [9] proved that anti-GPIIb α antibodies could activate Akt and induce platelet apoptosis through activation of phosphodiesterase (PDE3A) and PDE3A-mediated PKA inhibition. Either gene ablation or chemical inhibition of AKT [9] and PKA [10] molecules in the PI3K/AKT pathway can eliminate platelet apoptosis induced by anti-GPIIb α antibodies, prevent platelet phosphatidylserine (PS) exposure, and reduce platelet clearance. On the other hand, platelet autophagy, which selectively removes damaged mitochondria to reduce intracellular ROS concentration and prevent apoptosis, is considered an important self-protective mechanism for platelets [11]. In 2019, Chun-Yan Wang *et al* [12] reported that the level of autophagy was significantly reduced in ITP patients, and inhibition of PI3K activity could induce platelet autophagy to reduce the level of platelet apoptosis and reduce the clearance and destruction of platelets.

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In this study, network pharmacology studies were used to screen out two key active ingredients, quercetin, and kaempferol, from hundreds of Chinese herbal ingredients based on the basic TCM theory of treating ITP “from the spleen” underlined by the Jianpi Yiqi formula, and the characteristics of “mitochondrial dysfunction” of “spleen deficiency syndrome”. Molecular docking analysis revealed that quercetin and kaempferol could stably bind to PI3K/AKT to exert inhibitory effects. Moreover, the PI3K/AKT/mTOR axis was identified as a key signaling pathway for the regulation of apoptosis (the inhibition of the PI3K/AKT/mTOR pathway could inhibit apoptosis).

Therefore, we deduced that quercetin and kaempferol, which inhibited platelet apoptosis through superimposed inhibition of the PI3K/AKT/mTOR signaling pathway, might be the pharmacological mechanism for the treatment of ITP with the Jianpi Yiqi granules.

Chinese herbal compound formula is composed of multiple herbal medicines, which are characterized by complex chemical composition and a wide range of action targets. Due to the complexity of the TCM compound formula; there is a lack of scientific, rational, effective, and safe evaluation systems for the study of the pharmacological substance basis and mechanism of action of TCM. In this study, a simple prediction of the target sites and action pathways for the treatment of ITP with Jianpi Yiqi formula was made using a network pharmacological analysis database and a molecular docking platform. Consequently, the rationale for the clinical treatment of ITP and other diseases with Jianpi Yiqi formula was explained, providing a reference for the study of the mechanism of action of Jianpi Yiqi formula. However, these results are not supported by experimental data; thus, further investigation of the mechanism of action of the Jianpi Yiqi formula is required to provide further support for reported results.

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Authorship contributions

Qiming Li and Jiafeng Yang conceived and designed the experiments; Qiming Li, Gangdeng, Yunlei He performed the experiments and evaluated the results, Qiming Li and Jiafeng Yang wrote the manuscript.

Conflict of interest disclosure

All authors have no conflicts of the final version of the manuscript.

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