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A study on the mechanism of action of YiQiYangYin decoction for the treatment of diabetes based on network pharmacology

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ABSTRACT

To study the mechanism of action of YiQiYangYin decoction on diabetes mellitus by a network pharmacology method. The chemical components and targets of all the drugs in the YiQiYangYin decoction were obtained through the Traditional Chinese Medicine Systematic Pharmacology Database and Analysis Platform (TCMSP) and Traditional Chinese Medicine Integrated Database (TCMID), and the targets of diabetes were screened through the GeneCards and OMIM databases. The obtained targets were imported into Cytoscape 3.7.2 software to construct the active ingredient target network and were imported into the String database to construct the protein-protein interaction (PPI) network, and the Bisogenet plug-in in Cytoscape 3.7.2 was used for network topology analysis. Gene Ontology (GO) enrichment analysis and Kyoto gene and genomic KEGG enrichment analysis were performed on the potential targets of YiQiYangYin decoction for diabetes mellitus using the R language Bioconductor platform, and the results were imported into Cytoscape3.7.2 software to obtain KEGG network relationship maps. Molecular docking software AutoDock Vina was used to dock the core targets with the active ingredients. A total of 61 chemical components and 581 disease targets were obtained, including 1100 intersecting targets. The key targets included ALB, AKT1, and IL-6. GO functional analysis showed that BP was mainly involved in oxidative stress and response to lipopolysaccharide, epithelial cell proliferation, response to oxidative stress and ossification. MF was mainly involved in receptor-ligand activity, cytokine activity, cytokine receptor binding, nuclear receptor activity, etc. CC was mainly involved in the endoplasmic reticulum lumen, transcription factor complex, membrane raft, microfilm region, etc. KEGG enriched 159 signaling pathways, including the AGE/RAGE signaling pathway, TNF signaling pathway, and IL-17-mediated MAPK signaling pathway. The molecular docking results showed that quercetin and lignocaine had a good binding activity with AKT1 and ALB. The treatment of diabetes mellitus by YiQiYangYin decoction works through multiple components and targets, which lays the foundation for further study of its mechanism of action.

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Introduction

Diabetes mellitus (DM) is the most common metabolic disease of the endocrine system and is mostly seen in middle-aged and elderly people. China has the largest number of diabetic patients in the world (1). According to the data of the International Diabetes Federation in 2013, the total number of diabetic patients aged 20-79 years was 382 million worldwide, accounting for 8.3% of the total population in this age group, of which China accounts for 98 million, and it is estimated that the number will increase to 143 million in 2035 (2-4). The common causes of DM are mainly genetic, environmental, age and lifestyle (5,6). Currently, the treatment of diabetes mellitus is mainly through drugs, diet and exercise, and the search for natural active ingredients in Chinese herbal medicine for multitarget therapy has become a hot topic of research compared to single-target therapy (7). The decoction contains astragalus, mulberry leaf, yucca, geranium and propolis powder. It has the effect of benefiting and nourishing, clearing heat and detoxifying toxins. Some studies have shown that YiQiYangYin decoction has good effects in the treatment of chronic nephritis and diabetes.

Materials and Methods

Screening of target components of YiQiYangYin decoction

The TCM system pharmacology platforms TCMSP and TCMID were used to find the active chemical components of Chinese herbal medicines (Astragalus membranaceous, mulberry leaf, Yuzhu, Pueraria lobata, propolis powder) in the decoction of YiQiYangYin, and oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 were set in TCMSP for screening. The active ingredients of the herbal medicines in the decoction were obtained. The potential protein targets were obtained from the Swiss target prediction database with the screening condition probability ≥ 0.1, and the screened protein targets were converted into standardized gene names in the UniProt database.

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Diabetes disease-related target screening

Diabetes mellitus was used as a search term in the OMIM and GeneCards databases to obtain diabetes-related target genes. The number of targets was filtered according to the score value; the larger the score value, the stronger the association between the target and the disease, and the targets with a score greater than the median were usually set as potential targets for the disease, leaving the genes with a score greater than 5 in the genecard to be combined and deweighted with the OMIM database.

Acquisition of effective targets and drawing of a Venn diagram

The intersection of the targets of the herbal compound and the targets of the disease is obtained by using the Wayne diagram, and the intersection of the targets of the two is the effective target.

Active ingredient-active target network construction analysis

The active ingredients and the effective target genes were imported into Cytoscape 3.7.2 software for network construction and visualization analysis to obtain the drug-active ingredient-target network diagram, and the main active ingredients were screened by topological parameter analysis with the magnitude of degree (degree) value as the index.

Construction of protein networks

Through the GeneMANIA (http://genemania.org/) database, we imported the potential targets of YiQiYangYin decoction against diabetes into it, obtained the interactions between the targets, and then obtained the indirect targets. The indirect targets were added to the library of targets of action, imported into the String database, selected Homo sapiens, scored by default 0.4, and the target interaction network was obtained, saved in TSV format, imported into Cytoscape 3.7.2 software for topological analysis of the interaction network, and the node degree, betweenness, topological coefficient (TC) and closeness centrality (CC) of the nodes were obtained, and the top 3 targets were selected as key target proteins.

Enrichment analysis of target functions and pathways

We used R software (https://www.r-project.org/) and its backend database org.Hs.eg.db to obtain gene IDs (entrezID) of potential targets and then used DOSE, clusterProfiler, and the pathview package (Bioconductor) to perform GO functional enrichment analysis of these potential targets. The GO function enrichment analysis of these potential targets was performed by using DOSE, clusterProfiler, and pathview package (Bioconductor), including three aspects: biological process (BP), cellular component (CC), and molecular function (MF), setting pvalueCutoff = 0.05 and qvalueCutoff = 0.05. The GO enrichment analysis was divided into 3 main categories, including biological process (BP), molecular function (MF) and cellular component (CC). Each category is ranked according to significance, and the top 10 enrichment entries are presented in the form of bar and bubble charts.

Main active ingredient-target molecular docking of Yi QiYangYin decoction

The targets of YiQiYangYin decoction acting on diabetes were searched in the PDB database and saved in PDB format. Ligands were selected from the top 2 compounds in terms of degree value after topological analysis and saved in mol2 format. The potential targets of YiQiYangYin decoction for diabetes were molecularly docked with the main compounds in YiQiYangYin decoction using AutoDockTools -1.5.6.

Results

Acquisition of active ingredients and related targets of Yi QiYangYin decoction

In TCMSP and TCMID, 61 active ingredients in Yi QiYangYin decoction were obtained with OB ≥ 30% and DL ≥ 0.18, GI absorption as high in pharmacokinetics, and more than two yeses in drug-likeness as screening conditions, and the active ingredients are shown in Supplementary Table S1. A total of 505 Chinese medicine targets were retrieved.

Diabetes-related target acquisition

The disease genes were obtained from genecard and Omim, and the targets with scores greater than the median were empirically set as potential targets for the disease. Combined with the relevant targets obtained from the OMIM database search, duplicate values were merged and deleted, and finally, 581 diabetes-related targets were obtained.

Venn diagram

The Venn tool in TBtools was used to take the intersection of the YiQiYangYin decoction targets and the diabetes targets, and 100 intersection targets of both were obtained, and the results are shown in Figure 1.

Construction of the active ingredient-effective target network diagram of Yi QiYangYin decoction

Cytoscape 3.7.2 was used to construct the network of active ingredients and effective targets of Yi QiYangYin decoction, which is shown in Figure 2. The topological parameters of the network of YiQiYangYin decoction for the treatment of diabetes were calculated by the software and thus used to evaluate the importance of active ingredients and targets of action. The results revealed that active ingredients such as lignocaine and quercetin could act on multiple targets, and these ingredients might be the main active ingredients of YiQiYangYin decoction for the treat-

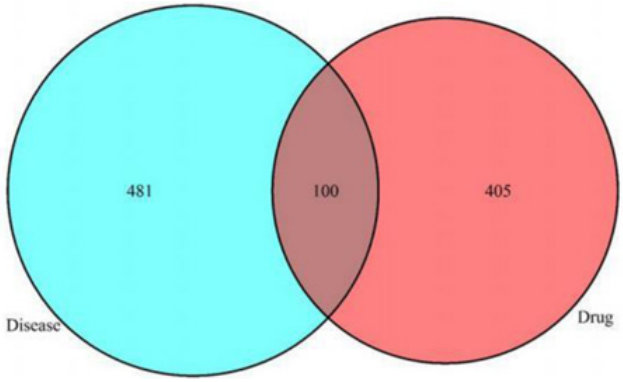

Figure 1. Venn diagram of the intersection target of Yi QiYangYin decoction and diabetes.

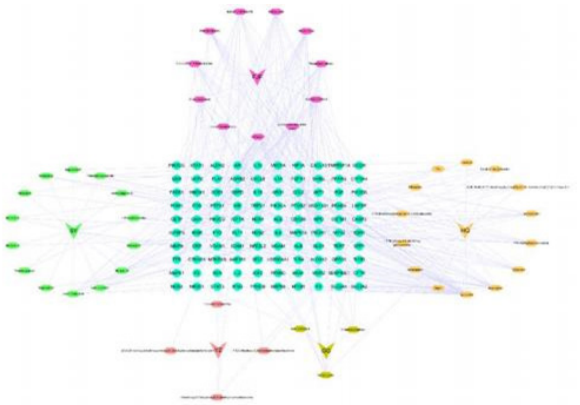

Figure 2. Active ingredient-active target network of YiQiYangYin decoction.

ment of diabetes mellitus.

Construction of protein networks

The intersection of the targets of YiQiYangYin decoction and diabetes mellitus was taken by the Venn tool (see Figure 1), and the intersection targets were uploaded to the STRING database with a confidence level ≥ 0.9 to obtain the PPI network map of the targets. The data were imported into Cytoscape 3.7.2 to draw the protein network relationship map. The larger the node, the larger the corresponding degree value and the position in the network was judged according to the degree value. According to Figure 3, the targets in the center of the network are TP53, AKT1, IL6, etc., which are presumed to be important targets for the treatment of diabetes mellitus with YiQiYangYin decoction.

Results of enrichment analysis of target functions and pathways

The GO annotation analysis of the validated targets was performed using R. The top 10 results of BP, CC, and MF were selected, and it was found that these target BPs were mainly involved in oxidative stress and response to lipopolysaccharide, epithelial cell proliferation, response to oxidative stress, and ossification. MF was mainly involved in receptor-ligand activity, cytokine activity, cytokine receptor binding, nuclear receptor activity, etc. CC was mainly involved in the endoplasmic reticulum lumen, transcription factor complex, membrane rafts, microfilm region, etc. The results are shown in Figure 4A.

KEGG enriched 138 signaling pathways analyzed, including the AGE/RAGE signaling pathway, TNF signaling pathway, IL-17-mediated MAPK and other pathways, and the top twenty of them were selected for visualization, and the results are shown in Figure 4B.

Molecular docking results of the active ingredients of Yi QiYangYin decoction

Using AutoDockTools -1.5.6, the potential targets of YiQiYangYin decoction in diabetes were molecularly docked with the main compounds of YiQiYangYin decoction calculated by the topological analysis, and the more stable the conformation of ligand-receptor binding, the higher the possibility of action, and the top-ranked key targets were selected according to the degree value. Using AutoDock Vina for molecular docking of the components, a binding energy < - 4.25 kcal/mol indicates some binding activity

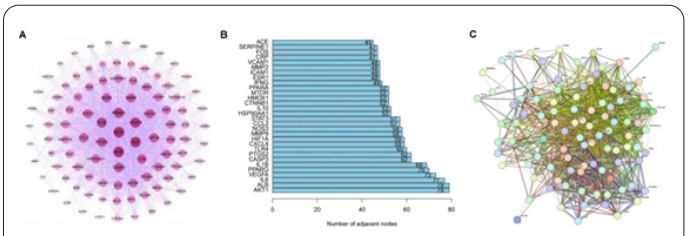

Figure 3. Intersection target PPI network diagram. Note: (A) PPI network map; (B) Core gene map; (C) Protein interaction map.

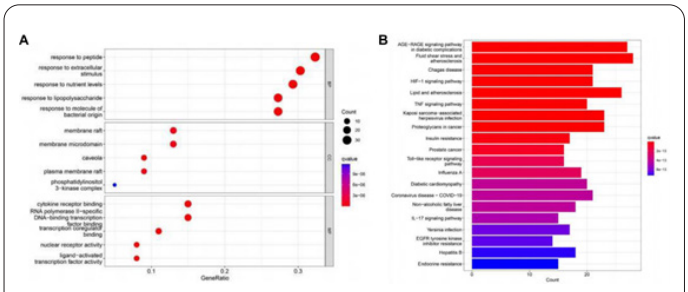

Figure 4. Enrichment analysis of beneficial YiQiYangYin decoction for diabetes mellitus. Note: (A) GO enrichment analysis; (B) KEGG enrichment analysis circle diagram.

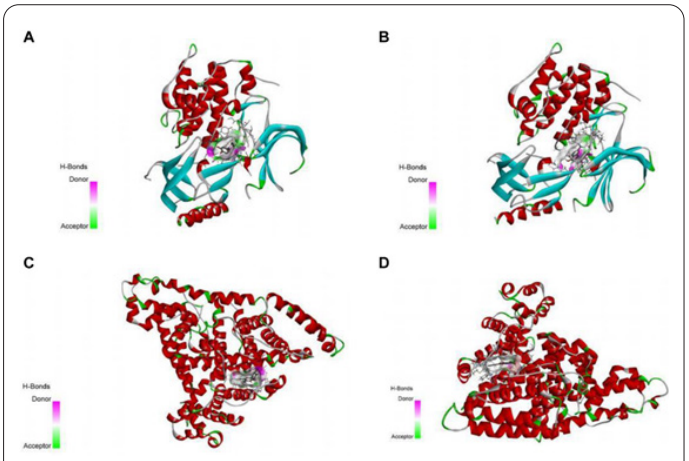

Figure 5. Molecular docking pattern diagram. (A) Docking diagram of quercetin and AKT1; (B) Docking diagram of lignocaine and AKT1; (C) Docking diagram of quercetin and ALB; (D) Docking diagram of lignocaine and ALB.

between the ligand small molecule and the receptor protein; a binding energy < -5.0 kcal/mol indicates good binding activity between the two; and a binding energy < -7.0 kcal/mol indicates that the ligand and the receptor have strong conjugation activity. The binding energies of quercetin and lignan AKT1 and ALB targets were -9.2, -9.3, -8.4, and -9.2 kcal/mol, respectively, indicating strong binding activity between the drug and the target pieces, as shown in Figure 5 for the specific docking results.

Discussion

In this study, using a combination of bioinformatics and network pharmacology, a total of 61 effective active ingredients were screened, corresponding to 505 active ingredient action targets, and 581 disease gene targets were intersected to obtain a total of 100 common targets, among which the active ingredients included quercetin and lignan. The results of this study provide a more comprehensive

discussion of the molecular mechanism of Yi QiYangYin decoction for the treatment of diabetes.

This study provides a more comprehensive discussion of the molecular mechanism of Yi QiYangYin decoction for the treatment of diabetes mellitus and provides a reference for the development of therapeutic drugs for diabetic diseases.

Through topological analysis of the ingredient-target-pathway network, active ingredients such as quercetin and muxuelein corresponded to more targets and were the key active ingredients for the treatment of diabetes mellitus. Based on the PPI network, AKT1, ALB and IL-6 were found to be the core targets of YiQiYangYin decoction for the treatment of diabetes mellitus. Serum ALB is known as an "intravascular transporter" and can bind to various ions, hormone molecules and drug molecules to participate in a variety of pathophysiological processes, in addition to stabilizing colloid osmotic pressure, anti-inflammatory and antioxidative stress (8,9). Studies have shown that the severity of hypoproteinemia is significantly associated with poor renal outcomes, while the risk of end-stage renal disease (ESRD) has been shown to increase significantly with decreasing serum ALB levels relative to patients with normal serum ALB (10,11). AKT1 is an important member of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, which is involved in the regulation of glucose homeostasis, lipid metabolism, and cell survival. AKT1 is an important member of the PI3K/Akt pathway that is involved in regulating glucose homeostasis, lipid metabolism, cell survival, angiogenesis, and other pathophysiological processes (12-14). It has been shown that decreased expression of phosphatase and tensin homologs (PTEN) in diabetic rat kidney tissue allows TGF-β1 to over-activate the PI3K/Akt pathway and promote the development of diabetes (15). Diabetes is closely related to chronic inflammatory processes, and both TNF and IL -6 inflammatory TNF and IL-6 are both inflammatory factors, and TNF can induce a series of inflammatory processes that cause damage to the glomerular filtration barrier (16). Studies have shown that serum IL-6 is positively correlated with the degree of proteinuria in diabetic patients and that high glucose stimulates IL-6 production in podocytes, thylakoid cells, mesenchymal tissue and tubules, promoting local and systemic inflammatory processes in diabetes (17-19).

Several important signaling pathways, including the AGE-RAGE, TNF, and IL-17D pathways, were predicted by KEGG enrichment fractionation, and these pathways may be important in the regulation of diabetes by the YiQiYangYin decoction. The AGE-RAGE signaling pathway is an important link in the development of diabetes and its complications, and AGEs are a heterogeneous group of nonenzymatic reaction products of aldehydes with proteins and lipids. Excessive deposition disrupts the structure of the extracellular matrix, alters its biochemical properties and metabolism, and leads to covalent cross-linking of proteins (20-22). In diabetic patients, AGE formation occurs on a large scale and may cause complications such as cataracts, atherosclerosis, diabetes and neuropathy (23, 24). RAGE is a well-studied receptor for AGEs and is an immunoglobulin RAGE consisting of an extracellular structural domain, a transmembrane structural domain, and a short cytoplasmic structural domain and is widely expressed in lung, heart, and kidney tissues. The binding of AGEs to their receptor RAGE activates a range of signaling pathways, including activation of protein kinases, JAK-STAT, PI3K-Akt, MAPK, and tyrosine phosphorylation of the calcium signaling pathway (25). By activating the MAPK and PI3K-Akt signaling pathways, NAPDH can be further activated to promote the formation of reactive oxygen species while enhancing oxidative stress, further stimulating the production of cytokines and growth factors that can cause damage to cells and tissues (26).

In conclusion, this study predicted the possible targets of YiQiYangYin decoction for the treatment of diabetic nephropathy and the biological processes and pathways that may be involved using network pharmacology and molecular docking techniques. This study provides some research ideas for subsequent basic research on the treatment of diabetes with YiQiYangYin decoction.

Conflict of Interests

The authors declared no conflict of interest.

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