Network Pharmacology Integrated Molecular Docking Reveals The Mechanism of Huanglian Wendan Decotion Against Metabolic Syndrome

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Research

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Abstract

**Background:** Metabolic syndrome (MetS) is a group of abnormalities which includes abdominal obesity, hypertension, insulin resistance, and dyslipidemia. Many clinical studies showed that Huanglian Wendan Decoction (HWD) is effective in the treatment of MetS.

**Methods:** In this study, the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database, STITCH database and Swisstargetprediction database were used to excavate the active components of HWD and to predict the potential targets. The key targets of MetS are screened through GeneCards database and DisGeNET database. Moreover, we obtained the overlapping target of HWD and MetS based on venndiagram of "R program". Next, we performed enrichment analysis for common-target network and protein-protein interaction (PPI) network. Afterwards, Cytoscape was used to construct the components-targets core network for HWD in treating MetS, and R program was used to perform GO-BP and KEGG pathway enrichment analysis on these core targets. Last, we further investigated our predictions of crucial targets by performing molecular docking studies with components of HWD.

**Results:** The component-target core network of HWD against MetS was screened to contain 22 active components, which corresponded to 77 core targets. Additionally, enrichment analysis suggested that targets of HWD against MetS were mainly clustered into multiple biological processes (response to nutrient levels, gland development, response to steroid hormone, cellular response to oxidative stress, reproductive structure development etc and related pathways (AGE-RAGE, AMPK, JAK-STAT, MAPK), indicating the underlying mechanisms of HWD on MetS. The results of molecular docking showed that beta-sitost, naringenin, berberine and baicalein bound well to IL6 and AKT1.

**Conclusion:** The network pharmacological strategy integrated molecular docking to explore the mechanism of HWD against MetS. Our present outcomes might shed light on the further clinical application of HWD on MetS treatment.

Introduction

Metabolic syndrome (MetS) is a complex group of metabolic disorders which includes abdominal obesity, hypertension, insulin resistance, and dyslipidemia[1,2]. MetS is a risk factor leading to type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [3]. Patients who fulfil the criteria of MetS have a three-fold increased risk of diabetes, and two-fold increased risk of CVD[4-5]. Epidemiological surveys show that MetS is a highly and increasingly prevalent medical condition[6], affecting well over 20% of the adult population in the USA[7], China[8], Europe[9], and the developing countries. The pathogenesis of MetS is still not completely clarified. Present studies have suggested that insulin resistance, adipose tissue dysfunction, chronic inflammation, oxidative stress, circadian disruption, microbiota, genetic factors might be involved in the pathogenesis of MetS[10]. The unknown pathogenesis has brought difficulties to the treatment, existing therapies to tackle various components of MetS are limited by various factors[11-
Traditional Chinese medicine (TCM) has unique advantages in treating MetS. In recent years, TCM has made good progress in the treatment of MetS[14-15].

Huanglian Wendan Decoction (HWD) is originated from “Liu Yin Bian Zheng”, consisting of eight herbs: Coptis Chinensis (Huanglian HL), Aurantii Fructus Immaturus (Zhishi ZS), Poria Cocos (Schw.) Wolf (Fuling FL), Citri Exocarpium Rubrum (Juhong JH), Zingiber Officinale Roscoe (Shengjiang SJ), Licorice (Gancao GC), Arum Ternatum Thumb (Banxian BX), Caulis Bambusae in Tania (Zhuru ZR)[16-17]. The effects of HWD on MetS have been validated by clinical practice in multiple years. However, the scientific basis as well as potential pharmacological mechanisms of HWD is still unclear, which has great limitations on the clinical effect of HWD[18].

Due to the large number of chemical components of Chinese medicines, the interaction of multiple Chinese medicines in the prescriptions, the long experimental period, and the heavy workload, it is impossible to comprehensively test the target of Chinese medicines, nor to verify the all components through experiments. Herein, in this study, a comprehensive approach (network pharmacology combined with molecular docking) was utilized to probe the pharmacological mechanisms of HWD against MetS and providing ideas for subsequent research.

**Materials And Methods**

**2.1. Screening of Potential Pharmacological Active Components and Targets of HWD**

TCMSP[19](http://lsp.nwu.edu.cn/tcmsp.php) is a unique platform for systematic pharmacology of Chinese medicine, which can capture the relationship among drugs, targets and diseases. In order to obtain the information about the components of HWD, “Huang Lian, Zhi Shi, Fu Ling, Ju Hong, Sheng Jiang, Gan Cao, Ban Xia, and Zhu Ru” were used as key words to search in the TCMSP database. Then, according to the principle of pharmacokinetics absorption, distribution, metabolism and excretion in vivo, the drug screening threshold was set as OB (oral bioavailability) $\geq 30\%$, DL (patent drug similarity) $\geq 0.18$. We also obtained potential targets protein names of compounds by TCMSP database. Then the candidate targets were mapped to Universal Protein[20] (Uniprot, http://www.uniprot.org/) for annotation and normalization. In addition, we also search Stitch database[21] (http://Stitch.embl.de/ver) and Swisstargetprediction database[22] (http://www.swisstargetprediction.ch/) to supplement the targets of the compounds obtained by TCMSP database. To ensure the accuracy of the targets, set the status of the targets in the TCMSP database to be validated, the probility of the target in swisstargetprediction is 1, and the combine~score of the target in the Stitch database is greater than 0.99.

**2.2 Collection of known MetS-related targets**

In order to obtain the known MetS-related targets, “metabolic syndrome” was used as key word to search in GeneCards database[23] and DisGeNET database[24]. After searching in the GeneCards database,
results were sorted by the relevance score (RS), following by the removal of targets lower than the 20. Additionally, MetS-related targets from DisGeNET database were sorted by the disease specificity index (DSI), following by the removal of targets lower than the median of DSI.

### 2.3. Excavation of Core Targets of HWD for Treating MetS and Construction of Core Networks

In order to obtain the candidate targets of HWD for treating MetS, we uploaded both potential targets of HWD and known MetS-related targets to the online Wayne diagram tool[25] ([http://bioinfogp.cnb.csic.es/tools/venny/index.html](http://bioinfogp.cnb.csic.es/tools/venny/index.html)) for overlapping targets. Subsequently, the candidate targets were used to construct a PPI network with multiple protein patterns on the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) platform[26] ([https://string-db.org/](https://string-db.org/)). We set the organism type to “Homo sapiens” and left the default settings in place for the other parameters. Then, we exported the “string_interactions.tsv” file. Then, Cytoscape 3.7.2 software was used to construct two types of networks: components-targets network, protein-protein interaction network.

### 2.4. Enrichment Analysis of Core Targets

GO-biological process (BP) and KEGG pathway enrichment analysis are important methods used to describe the characteristics of candidate targets. We selected the standard p_value cutoff of 0.05 and the q_value of 0.05 and performed the enrichment analysis with R programe[27](Bioconductor, clusterProfiler).

### 2.5. Molecular docking simulation

Combining disease-related pathways, find out the differential genes in the pathway, and then query the corresponding compound molecules, download the 3D structure from the RCSB protein database[28] ([http://www.pdb.org/](http://www.pdb.org/)). And use MOE software to modify it to remove ligands, add hydrogen, remove water, optimize and repair amino acids, and minimize the energy of all candidate targets. Finally, the above-mentioned core compound is used as a ligand, and the protein corresponding to the core target is used as a receptor for molecular docking.

## Results

### 3.1. Screening of Active Components and Their Targets of HWD

Retrieved from TCMSP database, there were 173 related components of the whole formula in total by the screen criteria of OB and DL. The active components of HL, ZS, FL, JH, SJ, GC, BX, were 14, 22, 15, 9, 5, 92, 13 respectively. 3 active components of ZR were retrieved from Stich database(ZR is not included in the TCMSP database, only through literature or searching other databases to find the compound of ZR). 134 active components remained after removing no target genes and duplication. Subsequently, we explored the potential targets of the 134 potential pharmacologically active components by excavating TCMSP databases, Stitch database and Swisstargetprediction database, which yielded to 219 targets after removing duplication(shown in Table S1).
3.2. Excavation of the Core Targets of HWD in Treating MetS

We collected 1728 targets (shown in Table S2) associated with MetS from the GeneCards database and DisGeNET database. The target genes of HWD were compared with the related genes of MetS and 77 overlapping common targets (shown in Table S3) were screened out. To explore the interaction between 77 overlapping common targets, we built a PPI network (Figure 1). The network had 76 nodes (one target had no interaction relationship with other targets, so the target was removed), which interacted with 817 edges, the average node degree is 21.5. As the degree increase, the nodes color deepens and size increase, the combine_score increase, the edges color deepens and thicker increase, which suggest stronger interactions. These results demonstrate that these targets were defined as the candidate targets for HWD in treating MetS.

3.3. Construction of Components-Targets Core Network for HWD in Treating MetS

In order to holistically and systemically obtain comprehensive understanding of the component-target for HWD in treating MetS, a network map was constructed by using Cytoscape. First, we found the corresponding components of 77 core targets. The basic information of the components of HWD is shown in Table 1. Then we used Cytoscape to construct a network map, including 301 edges and 115 nodes (Figure 2). It showed that HWD plays a therapeutic role in MetS mainly through multi-component corresponding to multi-target.

Table 1. active components of HWD.
| MOL ID    | Component name       | OB%  | DL  | Number of targets | Herb        |
|-----------|----------------------|------|-----|-------------------|-------------|
| MOL000098 | quercetin            | 46.43| 0.28| 84                | GC HL       |
| MOL000289 | pachymic acid        | 33.63| 0.81| 1                 | FL          |
| MOL000354 | isorhamnetin         | 49.6 | 0.31| 5                 | GC          |
| MOL000358 | beta-sitosterol      | 36.91| 0.75| 3                 | BX JH       |
| MOL000392 | formononetin         | 69.67| 0.21| 5                 | GC          |
| MOL000417 | Calycosin            | 47.75| 0.24| 1                 | GC          |
| MOL000422 | kaempferol           | 41.88| 0.24| 8                 | GC          |
| MOL000449 | stigmasterol         | 43.83| 0.76| 4                 | BX SJ       |
| MOL000497 | licochalcone a       | 40.79| 0.29| 2                 | GC          |
| MOL001454 | berberine            | 36.86| 0.78| 1                 | HL          |
| MOL001803 | sinensetin           | 50.56| 0.45| 1                 | ZS          |
| MOL001942 | isoimperatorin       | 45.46| 0.23| 4                 | JH          |
| MOL002670 | Cavidine             | 35.64| 0.81| 1                 | BX          |
| MOL002714 | baicalein            | 33.52| 0.21| 10                | BX          |
| MOL002776 | baicalin             | 40.12| 0.75| 1                 | BX          |
| MOL004328 | naringenin           | 22.05| 0.74| 24                | GC          |
| MOL004804 | 18beta-glycyrrhetinic acid | 59.29 | 0.21 | 1 | GC CP ZS JH |
| MOL005812 | naringin             | 22.05| 0.74| 1                 | GC          |
| MOL005828 | nobiletin            | 61.67| 0.52| 1                 | ZS CP       |
| arginine  |                      |      |     | 3                 | ZR          |
| glutamic acid |                    |      |     | 3                 | ZR          |
| ornithine |                      |      |     | 3                 | ZR          |

### 3.4. Enrichment Analysis of the Core Targets of HWD in Treating MetS

In order to further understand the mechanism of “multitarget and multipathway” of HWD in treating MetS, “R program” was used to perform enrichment analysis of GO-PB and KEGG on core targets and to excavate the biological processes and signaling pathways regulated by HWD in treating MetS. These 77 core targets were involved in several biological process, mainly including response to nutrient levels, gland development, response to steroid hormone, cellular response to oxidative stress, reproductive
structure development et al (Figure 4). Moreover, according to the p-values of enriched pathways and their correlation with MetS (shown in Table S4), we were most interested in the following five representative signal pathways including AGE-RAGE, MAPK, AMPK, JAK-STAT signaling pathways (Table 2).

Table 2 Representative enriched KEGG pathway of the core targets of HWD in treating MetS.

| Pathway                          | Gene count | P value   | Pathway ID | Associated genes |
|----------------------------------|------------|-----------|------------|------------------|
| AGE-RAGE signaling pathway       | 9          | 1.93E-07  | hsa04933   |                  |
| AMPK signaling pathway           | 8          | 9.39E-06  | hsa04152   |                  |
| JAK-STAT signaling pathway       | 8          | 8.22E-05  | hsa04630   |                  |
| MAPK signaling pathway           | 12         | 4.91E-08  | hsa04010   |                  |

3.5 Predicted binding of components of HWD to target proteins in MetS

To further validate candidate compounds of HWD targets in MetS, we tested the precision of docking between beta-sitost, naringenin, berberine, baicalein and the following potential target proteins (Figure 4-5): IL6 (PDB: 6NCO) and AKT1 (PDB: 5KCV). We chose to study these target proteins because they were high-degree nodes in the network with many functional connections. Meanwhile, there were enriched in related-pathways, suggesting they play a critical role in the response to compounds in MetS. Docking analysis successfully predicted docking between beta-sitost, naringenin, berberine, baicalein and the binding pocket of two tested target proteins. Overall, these results provide further evidence that these two proteins act as beta-sitost, naringenin, berberine and baicalein targets in MetS.

Discussion

HWD is an common prescription for MetS at the Department of Endocrinology in our hospital. It is especially suitable for patients with phlegm-heat subtype of MetS, with obesity, bitter mouth, dry mouth, red tongue, and yellow fur [29]. The therapeutic effects of HWD in the clinical treatment of MetS are significant [30]. However, the active compounds and potential targets of HWD are unclear, which hinders the further development and application of the prescription. In this study, network pharmacology combined with molecular docking approach were applied to determine the underlying mechanisms of HWD in MetS.

In this study, a total of 22 potential active components of HWD in treating MetS were screened through a series of network pharmacological methods. Beta-sitosterol, naringenin, quercetin, stigmasterol and nobiletin are common compounds of two or more Chinese medicines. Studies have shown that beta-sitosterol alone can increase plasma adiponectin concentration and reduced plasma insulin
concentration and homeostatic model assessment index[31]. Naringenin supplementation has proven to be efficacious for the treatment of metabolic syndrome and obesity in animal models[32]. Naringenin may be useful for ameliorating the inflammatory changes in obese adipose tissue. Recent researches have demonstrated berberine can protect rats from MetS through exerting inhibition on the HPA-axis and increasing skeletal muscle expression of GLUT4 proteins[33]. Research showed baicalein protects mice from MetS through an AMPKα(2)-dependent mechanism involving multiple intracellular signaling pathways[34]. The compound-target network diagram (Fig. 2) shows that there was a complex network relationship between the compounds and the targets.

In the PPI network, 77 core targets are important target proteins for the HWD as used to treat MetS. The top 5 targets (INS, IL6, AKT1, VEGFA, ESR1) ranked by degree have been confirmed to be closely related to the occurrence and development of MetS. INS gene mutations have been confirmed to be closely related to the occurrence of MetS[35]. Studies have shown that IL-6 is involved in the occurrence and development of all components in MetS such as hypertension, insulin resistance, obesity and lipid dysmetabolism[36]. AKT1 is a key mediator in the insulin-signaling pathway which has been proven to be an important target proteins for the HWD as used to treat MetS[37]. Recent studies have suggested that the VEGFA gene plays an important role in the pathogenesis of MetS and its related disorders. Targeting VEGFA and its inhibition and blockage have been suggested as novel therapeutic approaches to improve MetS.

Enrichment analysis of GO-BP and KEGG on the core targets further suggests that HWD could intervene in MetS through multiple biological processes by acting on several signaling pathways, involving AGE-RAGE, AMPK, JAK-STAT, MAPK signaling pathways. Luca Cannizzaro used samples taken from different body fluids and tissues of a rat model of high-fructose diet-induced MetS. The results showed that the following molecular parameters of the AGE-RAGE signaling pathway were significantly upregulated. Recent researches have demonstrated AMPK activation to ameliorate multiple components of metabolic syndrome by regulating a balance between anabolic and catabolic cellular reactions. Recent researches have demonstrated that dysregulation of the JAK-STAT pathway would lead to a multiple metabolism disorders and medicines for this signaling pathway maybe become a new idea for MetS. Animal experiment has shown spironolactone treatment administered inhibited phosphorylation of p38MAPK, which inhibit pancreatic gland β-cell apoptosis in MetS. This indicates P38MAPK pathways is the treatment target of MetS.

Conclusions

Our team has previously confirmed the safety and effectiveness of HWD in treating MetS through clinical observations. Network pharmacology and molecule docking method confirms the "multicompounds, multitargets" therapeutic actions of HWD in the treatment of MetS. The present work may provide valuable evidence for further clinical application of HWD for treating MetS and may lay a good theoretical foundation for further experimental verification and facilitate the widespread application of HWD in treating MetS.
**Abbreviations**

metabolic syndrome: MetS

cardiovascular disease: CVD

type 2 diabetes mellitus: T2DM

protein-protein interaction: PPI

Huanglian Wendan decoction: HWD

Traditional Chinese Medicine Systems Pharmacology Database: TCMSP

oral bioavailability: OB

druglikeness: DL

Search Tool for Interactions of Chemicals: STITCH

Universal Protein: Uniprot

Search Tool for the Retrieval of Interacting Genes/Proteins: STRING

biological process: BP

**Declarations**

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Disclosure**

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

Junli Bao designed the study, analyzed the data, and wrote the manuscript; Li Liu supervised the study and revised the manuscript; Yubo Han, Xinyuan Gao and Ke Zhang obtained and analyzed the data. All
authors have read and agreed to publish this manuscript.

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