The Mechanism of Simiao Powder in Treating Gouty Arthritis Based on Network Pharmacology

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Abstract: Gouty arthritis is a common clinical disease with high incidence and recurrence rates. The efficacy of Simiao Powder in treating gouty arthritis is reliable, but its mechanism of action is not yet clear. This project utilizes network pharmacology to explore the specific mechanism of action of Simiao Powder in treating gouty arthritis through molecular docking. It investigates the specific process of how traditional Chinese medicine treats diseases with multiple components, multiple targets, and multiple pathways. Thus, it explores potential targets and related mechanisms, providing experimental evidence for the clinical application of Simiao Powder in treating gouty arthritis and enriching the connotation of traditional Chinese medicine theory.

Keywords: Network Pharmacology; Simiao Powder; Gouty Arthritis;

1. Introduction

According to statistics, there are more than 100 million arthritis patients in my country. Among them, about 50% of people over the age of 65 suffer from varying degrees of arthritis, and arthritis has become one of the most common chronic diseases. October 12 is World Arthritis Day every year. It can be seen that the development of the treatment of gouty arthritis is urgent. Traditional Chinese medicine has long-term clinical practice and a lot of clinical experience in treating gout. Simiao Powder is often used in clinical practice to treat gout. Simiao Powder has many advantages in treating gout. Its ingredients are mostly natural drugs, with many targets and few adverse reactions. However, the research on the molecular mechanism of Simiao Powder in treating gout is not yet mature.

In recent years, network pharmacology has become a frontier and hot spot in the field of traditional Chinese medicine research. Traditional Chinese medicine [1] has the characteristics of multiple components, multiple targets, and multiple action paths. Its pharmacological material basis and mechanism of action are not yet clear, which is important for promoting the development of traditional Chinese medicine. There has been resistance. Internet pharmacology came into being. This research method can not only effectively predict the effective ingredients, targets of action and toxic and side effects of drugs, but also deeply study the relationship between metabolites and physiological pathology from the aspect of material metabolism, which is conducive to promoting the modernization of traditional Chinese medicine. Therefore, this project is based on previous research and deeply explores the molecular mechanism of Simiao Powder in treating gout through online pharmacology, and opens up new ideas for subsequent GA experimental pharmacotherapy research.

2. Materials and Methods

2.1 Screening of Active Pharmaceutical Ingredients

Using the TCMSP database (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform), we searched for the compounds of 'Atractylodes', 'Phellodendron', 'Achyranthes', and 'Coix Seed', and selected those with 'oral bioavailability (OB) ≥ 20%' and 'drug likeness (DL) ≥ 0.1' as the active ingredients of the traditional Chinese medicine. The corresponding targets for these active ingredients were then identified, and duplicate results were removed using Excel software.

2.2 Prediction of Active Ingredient Targets

UniProt was employed to query the gene names (protein IDs) of target proteins associated with the active ingredients of traditional Chinese medicine, with 'Homo sapiens' specifically selected as the search parameter. This step allows for the identification of the corresponding target gene names (GeneID), and any duplicates in the results are removed to ensure the accuracy and reliability of the data.

2.3 Prediction of Disease-Related Targets

Using the DisGeNET database, a search was conducted with "gout" as the keyword to identify disease-related targets associated with gout. During this process,data were meticulously filtered to remove duplicate targets and entries missing gene names, ensuring the uniqueness and completeness of the final set of targets.

2.4 Prediction of Potential Action Targets

Venny 2.1 software was used to perform an intersection analysis between the active ingredients of the drug and disease targets, aiming to identify potential targets for SMP in the treatment of gouty arthritis. By constructing a Venn diagram, the overlap between the drug and the disease is clearly illustrated, facilitating a deeper understanding of the mechanism of action of SMP in the treatment of this disease.

2.5 Construction of Protein-Protein Interaction (PPI) Network

In the STRING database, the species was limited to "Homo sapiens" to filter out protein-protein interactions (PPIs) with higher enrichment levels. This step allowed the construction of a core PPI network, clearly illustrating the significant interactions between proteins, which aids in the deeper understanding of their roles and regulatory mechanisms in biological processes.

2.6 Gene Ontology (GO) Enrichment Analysis

Using the DAVID6.8 database, gene ontology (GO) was performed on the core targets obtained from the PPI analysis of the intersection targets of Simiao Powder and gouty arthritis to obtain the main biological functions of Simiao Powder in treating gout. The analysis results were sorted according to p-value and the top 10 items were screened out.

2.7 Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis was performed on the potential targets identified through the Venn diagram. With a significance threshold of 'P < 0.01', the top 20 pathways were selected, and data were collected to construct the target-pathway relationship.

2.8 Construction of the "Drug-Target-Disease" Network

A functional association network was established between the active ingredient gene IDs and their effective targets, between the effective targets and key pathways, and between gout and effective targets of Simiaosan. The common results were then imported into Cytoscape 3.7.2. This process led to the construction of a 'drug-target-disease' network for Simiaosan's treatment of gout, providing valuable support and guidance for further research.

2.9 Molecular Docking

Before performing molecular docking [2], the active ingredients and core target protein structures were preprocessed. AutoDockTools was used to add hydrogen atoms and calculate the charge of the active ingredients, which were then saved in pdbqt format. For the core target protein structures, water molecules and some inactive regions were removed using PyMOL, followed by the addition of hydrogen atoms and charge calculation with AutoDockTools, and the files were saved in pdbqt format. The Lamarckian genetic algorithm was then applied to export the docking file, preparing for the subsequent semi-flexible docking step. Finally, PyMOL was used to visually present the molecular docking results.

3. Results

3.1 Screening Results of Active Ingredients in Drugs

A search of the database yielded 403 active compounds. Among these compounds, 49, 140, 176 and 38 appear respectively in Cangzhu, Huangbo, Niuxi, and Yiyiren. Next,

these active ingredients were screened under the conditions of "OB≥20%, DL≥0.1". The results showed that there were 28 in Cangzhu, 58 in Huangbo, 37 in Niuxi, and 18 in Yiyiren. These are the effective active ingredients of traditional Chinese medicine.

3.2 Prediction Results of Active Ingredient Targets

The target gene name (GeneID) corresponding to the target protein was obtained through UniProt, and a total of 1543 targets were retrieved, including 118 Cangzhu, 766 Huangbo, 505 Niuxi, and 154 Yiyiren. After deleting duplicate results using Excel software, it was found that there were 293 predicted targets for active ingredients in the four traditional Chinese medicines.

3.3 Prediction Results of Disease-Related Targets

Using "gout"(Goutyarthritis) as the keyword, the DisGeNET database was used to search and screen for known disease targets, and a total of 206 disease targets were collected.

3.4 Prediction Results of Potential Targets of Action

Using the Venny2.1.0 online platform, the targets of action of the active ingredients of Simiao Powder were compared and analyzed with the targets of gout related diseases. During this process, it was found that the active ingredients of Simiao Powder involved a total of 293 targets, while gout related diseases had 206 targets. After intersection analysis,14 potential targets were obtained, which may be the key to Simiao Powder's treatment of gout. See Figure 1.

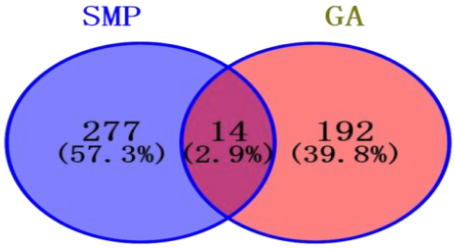

Figure 1. SMP Active Ingredient Targets and GA Disease Targets

3.5 PPI Network Construction

After screening, 14 nodes were identified as key targets, including key targets such as interleukin IL-6 (IL6), catalase proliferator activated receptor gamma (PPARG), interleukin IL1-β (IL1-B), and matrix metalloproteinase 9 (MMP9). See Figure 2, 3.

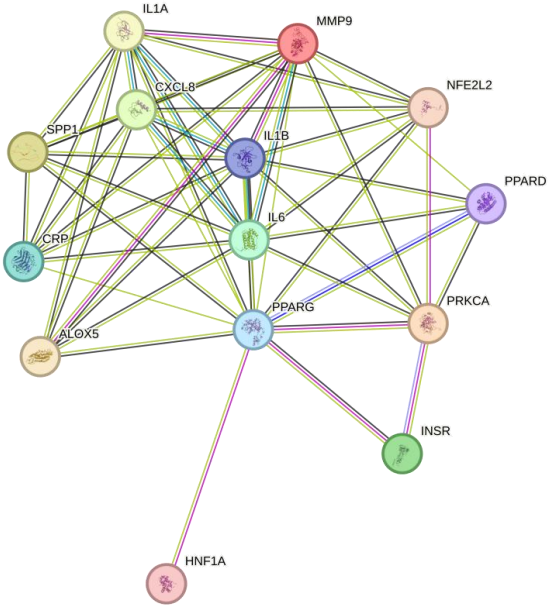

Figure 2. Topology Analysis of PPI Network

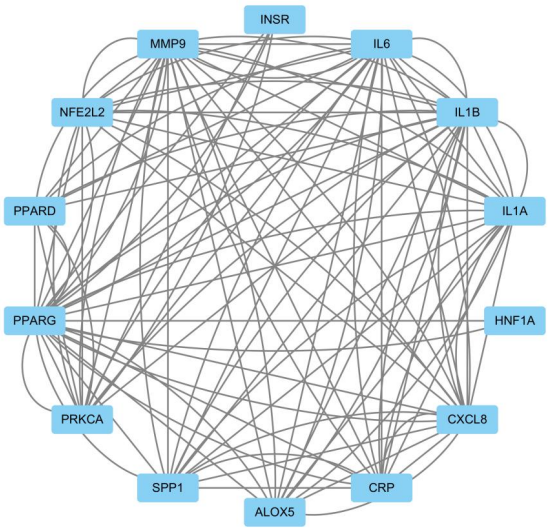

Figure 3. PPI Network Construction

3.6 GO Enrichment Analysis Results

Using the DAVID6.8 database, Geneontology (GO) functional analysis was carried out on the core targets obtained from PPI analysis of the intersection targets of Simiao Power and gouty arthritis, and the main biological functions of Simiao Power in treating gout were obtained. The GO functional analysis included BP (Biological process), CC (Cellular component), and MF (Molecular function). The analysis results were sorted according to p-value, and the top 10 items were screened out.

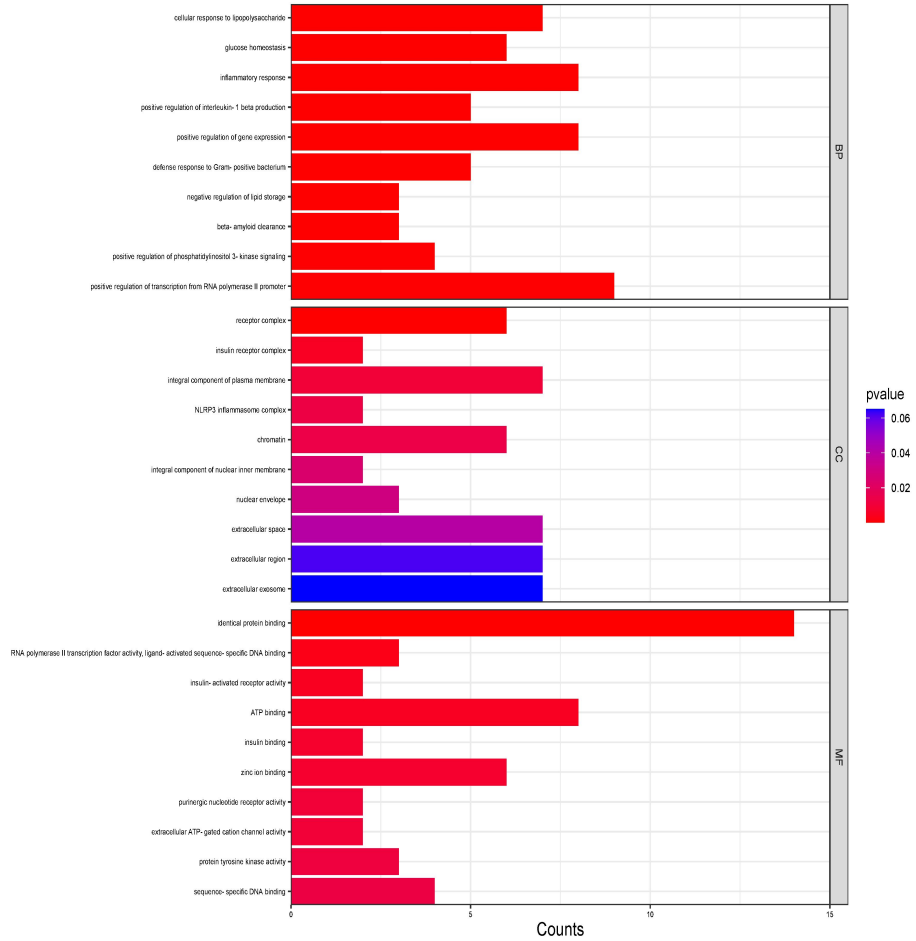

Figure 4. Go Enrichment Analysis

The GO results are as shown in Figure 4. The higher the column pattern, the more target enrichment is in this path; the redder the column pattern, the higher the enrichment of target substances in this path. Biological processes include cell response to lipopolysaccharide, glucose homeostasis, inflammatory response, etc.; Cellular components mainly involve receptor complexes, insulin receptor complexes, plasma membrane integral components, NLRP3 inflammatory complex, etc.; Molecular functions involve protein binding, RNA polymerase II transcription factor activity, DNA binding, etc.

3.7 KEGG Enrichment Analysis Results

The KEGG enrichment results are shown in Figure 5. The picture shows that the redder the bubble color and the smaller the p-value, the higher the target content on this path; the larger the bubble, the more targets there are on this path. It can be seen that SMP treatment of GA is mainly achieved by regulating HIF-1, alcoholic liver disease, Yersinia infection, influenza A, lipids and atherosclerosis, and NOD-like receptors. The effects of these signaling pathways may have a common effect, providing multi-faceted therapeutic effects for SMP in the treatment of gouty arthritis, helping to reduce gout symptoms and improve patients 'quality of life.

3.8 Construction of the "Drug Target Disease" Network

Establish the relationship between the active components-targets, disease-targets and intersection targets of Simiaosan, and then import them into Cytoscape 3.7.2 to construct a visual network of the relationship between "drug-target-disease" of Simiao Power in treating gout. The disease target is represented by the blue circular node on the right, the active ingredients are represented by the circular node on the left, the intersection of the active ingredients of Cangzhu, Huangbo, Niuxi, and Yiyiren is represented by the yellow square node, and the effective active ingredients are represented by the red circular node.

3.9 Molecular Docking Results

Based on the above obtained active ingredients and core targets, the top 20 active ingredients in degree were screened out, and the top three active ingredients were selected. Then the mol2 structures of the active ingredients were searched in TCMSP, which were Quercetin , Stigmasterol, Wogonin ; the top 3 core targets in degree were screened out to select the protein crystal structures with high crystal structure resolution in the protein structure-related database PDB (protein databank), and stored in file formats using the PDB standard, namely IL6, PPARG, and MMP9. Finally, Pymol was used for molecular docking visualization. Figure 6 for docking results.

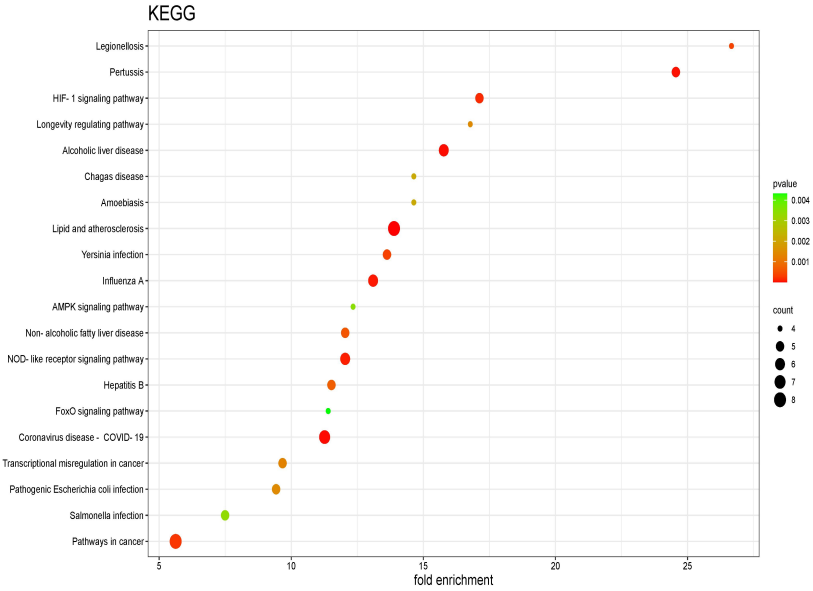

Figure 5. Kegg Enrichment Analysis

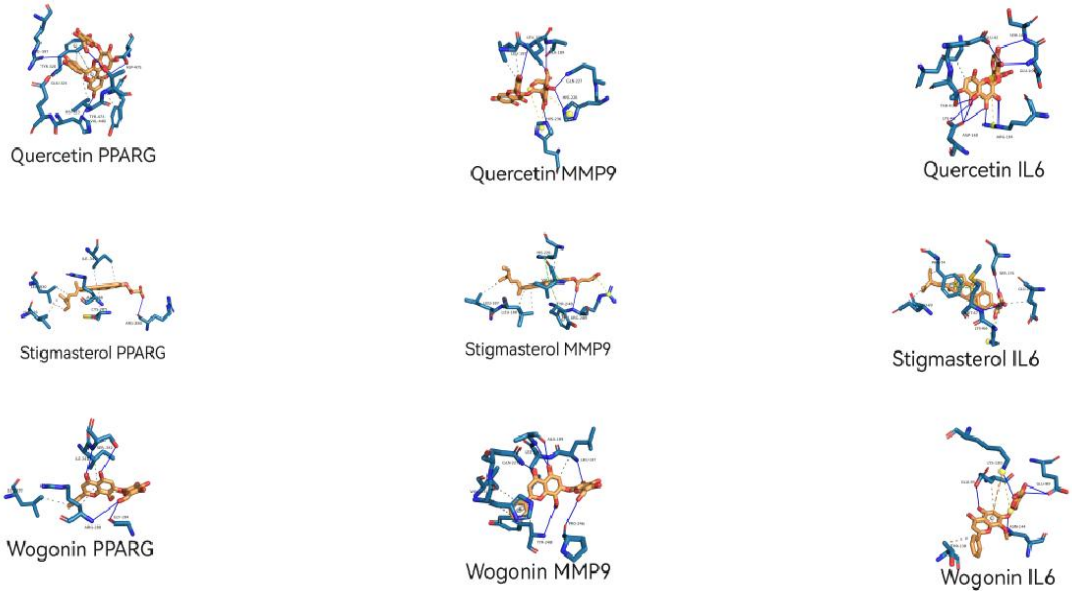

Figure 6. Molecular Docking

4. Discussion

Gouty arthritis [3] is a metabolic disease. The disorder of purine metabolism in the body and the decrease in uric acid excretion lead to the deposition of urate in the joints and the production of uric acid crystals, which stimulates the immune response and causes symptoms such as joint redness, swelling, heat, and pain. Traditional Chinese medicine believes that gout [4] is mainly caused by the obstruction of qi and blood in the body, and the obstruction of dampness and dampness and turbid phlegm flowing into the joints. Doctors in the past dynasties have often used Simiao Powder to treat gouty arthritis, and

achieved remarkable clinical results, demonstrating its important position in the treatment of traditional Chinese medicine. Simiao Powder [5-6] is composed of four traditional Chinese medicines: Huangbo, Cangzhu,Niuxi and Yiyiren. The combination of Huangbo and Cangzhu helps to clear heat and eliminate dampness, that is, eliminate moisture and heat in the body; the role of Niuxi is mainly to nourish liver and kidney, strengthen muscles and bones, and guide Cangzhu and Huangbo bark into the lower jiao, which helps these two drugs to better exert their effects in clearing heat and dampness, allowing them to act on the lower part of the human body. Most of the ingredients of Simiao Powder are natural drugs, with many targets and few adverse reactions. However, the research on the molecular mechanism of Simiao Powder in treating gout is not yet complete. Therefore, this topic plans to further carry out research on the molecular mechanism of Simiao Powder in treating gout based on previous research.

Constructing a "drug-target-disease" network and conducting biological activity enrichment analysis and molecular docking, it was found that Simiao Powder is composed of three main components: quercetin, stigmasterol,and wogonin. Figure 6 shows that the molecular docking results act on 14 targets including IL6, PPARG, IL1- β, MMP9, ALOX5, CRP, HNF1A, INSR, PRKCA, PPARD, NFE2L-2, CXCLB, IL-1A, SPP1, etc.

Studies [7] have shown that quercetin significantly reduces the levels of inflammatory factors TNF-α and IL-1β in diabetic peripheral neuropathy DPN rats. This decrease may be mediated by down-regulating the TLR4/MyD88/NF-κB signaling pathway, indicating that quercetin plays an important role in the regulation of inflammation. In addition, quercetin [8] can inhibit MSU-induced mechanical hyperalgesia, reduce inflammatory reactions, recruit white blood cells and enhance the production of TNFα, IL-1β and superoxide anions, and simultaneously play anti-inflammatory and analgesic effects.

Stigmasterol [9-10] is a plant sterol with multiple benefits, including lowering plasma cholesterol, anti-inflammation, anti-diabetes, anti-cancer, anti-oxidation, etc. Research by GABAY [11] and others has found that Stigmasterol can inhibit a variety of pro-inflammatory and matrix-degradation mediators involved in osteoarthritis induced cartilage degradation, part of which is achieved by inhibiting the NF-κB pathway. Similarly, MORGAN [12] et al. confirmed through experimental research that the interaction between the oxygen and nitrogen atoms of stigmasterol and the hydrogen bond between the residue and the binding residue arginine-611 can stabilize its anti-inflammatory mechanism.

Wogonin [13] is a flavonoid with multiple biological activities. It can inhibit inflammatory mediators produced by macrophages and lymphocytes. It can also [14] inhibit the NF-KB signaling pathway, reduce the inflammatory response mediated by NF-KB and p65, and inhibit apoptosis. In addition, Park et al. [15] used osteoarthritis chondrocytes to test the anti-inflammatory and chondroprotective effects of wogonin. The results showed that wogonin can reduce the level of inflammatory factors and inhibit the expression of MMP3 protein in articular chondrocytes. This shows that wogonin plays an important role in the treatment of gouty arthritis.

From the above results and analysis, it can be seen that the core targets of SMP in the treatment of GA are IL1-β, IL-6, MMP9, PPARG, PPARD, NFE2L-2, etc. Through [6] response to lipopolysaccharide, immune response, inflammatory response, and transcriptional regulation of RNA and DNA genes, it reduces the external apoptosis signaling pathway of ligands, and also plays a role in treating gout by participating in the regulation of sugar and drug metabolism pathways, such as diabetes, MAPK signaling pathway, NF-kB signaling pathway, NOD-like receptor signaling pathway, etc.

5. Conclusion

By studying the material basis of the anti-gout pharmacodynamic effect of Simiao Powder, it was found that its components have a close synergistic relationship with their targets, and the binding effect is good. This study will provide a theoretical basis for the treatment of gouty arthritis, lay a foundation for the application of Simiao Powder in the study of the pathogenesis of gout, and provide

evidence-based evidence for the modernization of traditional Chinese medicine.

Acknowledgments

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