Study on the pharmacodynamic material basis of network pharmacology of "Duhuo-sangjisheng" pair in the treatment of Rheumatoid arthritis

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Abstract: *Rheumatoid arthritis (RA) is a chronic systemic disease with unknown etiology, mainly inflammatory synovitis. Based on the theory background of Duhuojisheng Decoction in "BeiJiQianJin Yaofang",this paper takes Duhuo Jisheng decoction as the theoretical background, and develops the network pharmacology pharmacodynamic material basis of its compatibility in the treatment of rheumatoid arthritis. Firstly, TCMSP database was used to sort out the effective components and target genes. Next, gene cards database was used to collect disease-related genes, and then a four-dimensional relationship network of "drug-active ingredient-target gene-disease" was established. Finally, PubChem database was used to sort out the predicted components and summarize the pharmacodynamic basis.To some extent, it shows the significance of Duhuo Jisheng Decoction in the treatment of rheumatoid arthritis.*

Keywords: *"Duhuo-Sangjisheng" pair; network pharmacology; Pharmacodynamic material basis*

Introduction

In modern medicine, rheumatoid arthritis (RA) is widely recognized as a complex autoimmune disease characterized by chronic, symmetrical, and erosive polyarthritis. This condition not only affects joints but can also lead to a series of extra-articular symptoms, severely impacting the quality of life and health of patients. The etiology of RA is not completely understood, but studies suggest it may be related to genetic, environmental factors, and immune system abnormalities. Pathological features include synovitis and destruction of joint cartilage and bone. The goal of RA treatment is to reduce joint inflammation, inhibit disease progression and irreversible bone destruction, protect joint and muscle function as much as possible, and ultimately achieve complete remission or reduce disease activity. In traditional Chinese medicine (TCM), RA is defined as wind-cold-dampness bi syndrome, with commonly used prescriptions including "Du Huo Ji Sheng Tang."

1 Research Background

1.1 Etiology and Pathogenesis Analysis Based on the AP-1/NF-κB Signaling Pathway

In the pathogenesis of rheumatoid arthritis (RA), cytokines and inflammatory signaling pathways play a crucial role. Among them, AP-1 (Activator Protein 1) and NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) are two significant transcription factor families

involved in regulating the expression of various inflammation-related and immune response genes. AP-1 consists of multiple proteins, including c-Fos, c-Jun, and ATF family members, which form heterodimers or homodimers and bind to specific DNA sequences (AP-1 binding sites) to regulate target gene expression. In RA, the activation of AP-1 is associated with the production of inflammatory mediators, cell proliferation, and apoptosis.[1]

1.2 Analysis of the Ingredients of Du Huo Ji Sheng Tang

Du Huo Ji Sheng Tang is commonly used in clinical practice to treat rheumatoid arthritis and other diseases. The formula comprises 15 traditional Chinese medicines: Du Huo (9g), Sang Ji Sheng, Du Zhong, Niu Xi, Xi Xin, Qin Jiao, Fu Ling, Rou Gui Xin, Fang Feng, Chuan Xiong, Ren Shen, Gan Cao, Dang Gui, Shao Yao, and Gan Di Huang (each 6g). Du Huo, being the principal herb, is used in the highest amount of 9 grams. Du Huo, with its pungent, bitter, and slightly warm properties, is known for dispelling wind and dampness, unblocking the meridians, and relieving pain, particularly effective in treating wind-cold-dampness in the lower body and muscles and bones. Sang Ji Sheng, used as an adjuvant herb at a dosage of 6 grams, strengthens the muscles and bones and aids Du Huo in dispelling wind and dampness.

Du Huo and Sang Ji Sheng frequently appear as a herb pair, widely applied in treating rheumatoid arthritis. Through modern network pharmacology research methods, a four-dimensional network relationship diagram of active ingredients and target genes of the "Du Huo-Sang Ji Sheng" herb pair can be established. Using this relationship network, the pharmacodynamic substance basis related to the disease can be screened and explained, providing strong evidence to guide clinical practice and new drug development.[2]

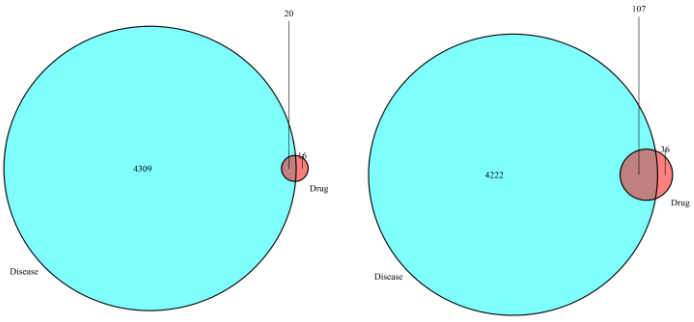
1.3 Databases Used

The information on the components and related targets of "Du Huo-Sang Ji Sheng" is sourced from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), while disease-related targets are obtained from the GeneCards database.[3]

2 Intersecting Genes

When screening active ingredients of the herbs using TCMSP, two conditions are typically selected: DL (Drug-Likeness) ≥ 0.18 and OB (Oral Bioavailability) ≥ 30%. In this study, only the DL value is considered while screening, ignoring the OB value, because active ingredients with low oral bioavailability still have research value. When these ingredients are applied in the future, other methods can be used to enhance their bioavailability to achieve the desired effect.

The target genes of the active ingredients of the herbs are intersected with the disease target genes to identify the active ingredients related to the disease, forming the pharmacodynamic substance basis of the herbs for treating the disease. The study of herb pairs is based on single herbs, and the results take the union of the intersecting genes. As shown in Figure 2-1, there are 20 intersecting genes between Du Huo and RA, and 107 intersecting genes between Sang Ji Sheng and RA.

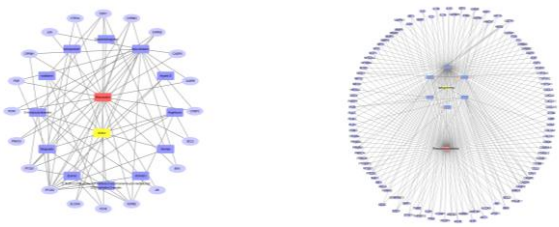


Venn diagram alone *Mulberry Venn diagram*

Disease-related gene *Drug-related gene*

figure（2-1）

Using the intersecting genes, a four-dimensional network relationship diagram of "drug-active ingredient-target gene-disease" is established, as shown in Figure 2-2. In the network relationship diagram, the "active ingredients" associated with the intersecting genes represent the pharmacodynamic substance basis of the drug for the disease. This network relationship diagram clearly displays the active ingredients linked to the intersecting genes, which are the pharmacodynamic substance basis of the drug for the specific disease. This visualized network diagram not only helps to reveal the molecular mechanisms of the drug's action but also provides important references for the clinical application and new drug development. Through this method, researchers can systematically understand how drug components influence disease treatment at the molecular level, thereby optimizing drug formulations and enhancing therapeutic effects.[4]



Radix angelicae network *Parasitic loranthus network*

medicine *Active ingredient* *Target gene* *illness*

figure（2-2）

As shown in Table 2-1, the active ingredients in the network diagram are organized, and the summary table is combined with information from the PubChem database as follows:

Pharmacodynamic ingredient list（2-1）

Plant origin	Active ingredient	Molecular formula	PubChem Cid
Radix angelicae*Radix Angelicae Biseratae*	Ammidin	$C_{16}H_{14}O_4$	10212
Radix angelicae	isoimperatorin	$C_{16}H_{14}O_4$	68081
Radix angelicae	Ammijin	$C_{20}H_{24}O_9$	216283
Radix angelicae	Zosimin/Columbianadin	$C_{19}H_{20}O_5$	6436246
Radix angelicae	Sitogluside	$C_{35}H_{60}O_6$	5742590
Radix angelicae、Parasitic loranthus	beta-sitosterol	$C_{29}H_{50}O$	222284
Radix angelicae	O-Acetylcolumbianetin/Columbianetin acetate	$C_{16}H_{16}O_5$	161409
Radix angelicae	Angelol D	$C_{20}H_{24}O_7$	5318740
Radix angelicae	Angelol G	$C_{20}H_{24}O_7$	10362168
Radix angelicae	Angelicone/Glabralactone	$C_{16}H_{16}O_5$	616303
Radix angelicae	Dipiperityl magnolol	$C_{38}H_{50}O_2$	101612410
Radix angelicae	nodakenin	$C_{20}H_{24}O_9$	73191
Parasitic loranthus *Herba Taxilli*	oleanolic acid	$C_{30}H_{48}O_3$	10494
Parasitic loranthus	Lupeol	$C_{30}H_{50}O$	259846
Parasitic loranthus	Sitosterol	$C_{29}H_{50}O$	222284
Parasitic loranthus	Quercitrin	$C_{21}H_{20}O_{11}$	5280459
Parasitic loranthus	Quercetin	$C_{15}H_{10}O_7$	5280343
Parasitic loranthus	Guajavarin	$C_{20}H_{18}O_{11}$	5481224

3 Effects of Predicted Active Ingredients in Rheumatoid Arthritis

3.1 Anti-inflammatory Effects

Ammidin (imperatorin) influences the apoptosis of RA synovial cells through the mitochondrial/caspase-mediated pathway. Isoimperatorin (IMP) has anti-inflammatory, analgesic, and antispasmodic effects, effectively downregulating Th2 cytokines to inhibit NF-κB and MAPK signaling pathways. Columbianadin/Columbianetin acetate has anti-inflammatory and analgesic properties,

inhibiting the NF-κB signaling pathway at multiple levels and targets. Sitogluside (β-sitosterol glucoside) has been shown to inhibit osteoporosis and may have a similar pathway inhibitory effect. Ursolic acid and beta-sitosterol effectively inhibit the MAPK, TNF-α, NF-κB, and Toll-like receptor pathways, inhibiting osteoclast differentiation. Oleanolic acid (OA) has anti-inflammatory, analgesic, and antitumor activities, inhibiting type II collagen and pro-inflammatory activities by increasing COX-2 expression. Angelol B, D, and G can inhibit platelet aggregation, disrupting the vicious cycle of the inflammation-coagulation network. Glabralactone downregulates inducible nitric oxide synthase (iNOS), inhibiting the LPS-activated NF-κB inflammation pathway. Lupeol has a high inhibitory effect on the production of inflammatory mediators PGE2, TNF-α, and IL-1β. Quercetin and quercitrin have excellent anti-inflammatory effects, with quercitrin's anti-inflammatory action in vivo involving the release of quercetin and inhibition of the NF-κB signaling pathway. Guajavarin (guava flavonoid) has anti-ulcer, conjunctivitis, and gingivitis effects.

3.2 Inhibition of Osteoporosis

Sitogluside (β-sitosterol glucoside) has been shown to inhibit osteoporosis. Due to its structural similarity to β-sitosterol, it may have a similar pathway inhibitory effect.

3.3 Antitumor Effects

Dipiperitylmagnolol has inhibitory activity against three types of small cell lung cancer. Oleanolic acid (OA) has anti-inflammatory, analgesic, and antitumor activities.

3.4 Regulation of Macrophage Function

In mice with arthritis, β-sitosterol can alleviate ankle swelling symptoms, reduce collagen-specific antibodies, and inhibit the production of pro-inflammatory cytokines. Due to its ability to regulate macrophage function, it has great potential as a new drug for treating RA.

3.5 Anti-allergic Inflammatory Effects

In a study by Lee N Y et al. on the anti-allergic inflammatory effects of PMACI-induced human mast cells (HMC-1), Nodakenin (peucedanol) was found to inhibit mRNA expression and the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1. Nodakenin also inhibited the nuclear translocation of the NF-κB signaling pathway and increased caspase-1 levels and IKK levels in PMACI-induced human mast cells (HMC-1).[5]

Besides these examples, GO and KEGG enrichment analyses revealed that effective components exert their effects through multiple pathways, including the NF-κB inflammation pathway and the MAPK signaling pathway. Additionally, network pharmacology analysis of all the herbs in the "Duhuo Jisheng Decoction" and RA found that herbs with liver and kidney tonifying and bone-strengthening effects, such as Duhuo, Sangjisheng, and Niuxi, have many intersection genes with RA. These herbs have many targets and broad pharmacological pathways, suggesting that tonifying the liver and kidneys and strengthening bones play a crucial role in treating rheumatoid arthritis, warranting further research and exploration.

4 Conclusion

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint inflammation and bone destruction, leading to joint pain, swelling, and functional impairment. This disease not only affects the quality of life of patients but can also lead to complications such as cardiovascular and pulmonary diseases. Therefore, the treatment and management of RA require a comprehensive approach, including medication, lifestyle adjustments, physical therapy, and psychological support.

Recent advances in understanding the pathogenesis of RA have revealed that various natural compounds exhibit significant efficacy in regulating RA-related signaling pathways. These compounds exert anti-inflammatory, analgesic, and immunomodulatory effects by influencing key pathways such as NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), MAPK (mitogen-activated protein kinase), and TNF-α (tumor necrosis factor-alpha). Compounds such as Ammidin, Isoimperatorin, Columbianadin, Sitogluside, Ursolic acid, Beta-sitosterol, Columbianetin acetate, Angelol B, D, G, Glabralactone, Dipiperitylmagnolol, Nodakenin, Oleanolic acid, Lupeol, Quercetin, Quercitrin, and Guajavarin have shown potential therapeutic value for RA.

The mechanisms of action of these natural compounds are complex and diverse. On one hand, they can reduce the inflammatory response in RA patients by inhibiting the NF-κB signaling pathway, thereby decreasing the production of inflammatory cytokines. NF-κB is a crucial transcription factor that regulates the expression of various inflammatory cytokines such as TNF-α, IL-1β, and IL-6. On the other hand, the MAPK signaling pathway plays an important role in the inflammatory response of RA. Activation of the MAPK pathway promotes the release of inflammatory cytokines and the synthesis of cellular cytokines, exacerbating RA. Therefore, by inhibiting the MAPK pathway, these natural compounds can slow down the progression of RA.

Additionally, these natural compounds may offer new strategies for RA treatment by affecting apoptosis, osteoporosis, and platelet aggregation. Apoptosis is a critical factor in RA joint damage, and excessive apoptosis leads to the destruction of joint cartilage and bone. Osteoporosis is a common complication in RA patients, severely affecting their bone health. By regulating these pathways, these natural compounds may help protect joint cartilage and reduce bone destruction, thereby improving the prognosis of RA patients.[6]

In traditional Chinese medicine, formulas such as Duhuo Jisheng Decoction have shown significant value in the comprehensive treatment of RA. These formulas, through their multi-target and multi-pathway mechanisms, can effectively alleviate joint pain and inflammation in RA patients. For example, Duhuo and Sangjisheng in the Duhuo Jisheng Decoction can modulate the immune system and inhibit inflammatory responses, thus reducing RA symptoms. Moreover, these formulas can improve the overall health of patients, enhancing their quality of life.

These research findings not only enrich RA treatment methods but also provide new ideas and directions for future drug development and clinical applications. With further understanding of the RA pathogenesis, researchers can design more effective drugs that comprehensively control RA by targeting multiple pathways and mechanisms. Additionally, combining traditional Chinese medicine with Western medicine can leverage the strengths of both medical systems, offering more comprehensive and effective treatment plans for RA patients.

In summary, the treatment and management of RA is a complex process that requires considering

multiple factors. By studying the mechanisms of natural compounds and traditional Chinese medicine formulas, researchers can develop new strategies for RA treatment. These strategies not only help alleviate RA symptoms and improve the quality of life of patients but also provide important scientific evidence for future drug development and clinical applications. With continuous advancements in science and technology, it is believed that RA treatment will become more effective, leading to a more optimistic prognosis for patients.

Fund Project

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References

[1] Wijerathne C U B , Seo C S , Song J W , et al. Isoimperatorin attenuates airway inflammation and mucus hypersecretion in an ovalbumin-induced murine model of asthma[J]. International Immunopharmacology, 2017, 49:67.

[2] Zhang W, Xue K, Gao Y, et al. Systems pharmacology dissection of action mechanisms of Dipsaci Radix for osteoporosis[J]. Life sciences, 2019, 235: 116820.

[3] Comalada M, Camuesco D, Sierra S, et al. In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway[J]. European journal of immunology, 2005, 35(2): 584-592.

[4] Choi J K, Kim S W, Kim D S, et al. Oleanolic acid acetate inhibits rheumatoid arthritis by modulating T cell immune responses and matrix-degrading enzymes[J]. Toxicology and applied pharmacology, 2016, 290: 1-9.

[5] Liu Rui,Hao Donglin,Xu Wenya,Li Jinjin,Li Xiaoru,Shen Dong,Sheng Kang,Zhao Lin,Xu Weiwei,Gao Zhongen,Zhao Xu,Liu Qiuhong,Zhang Yiting. β-Sitosterol modulates macrophage polarization and attenuates rheumatoid inflammation in mice.[J]. Pharmaceutical biology,2019,57(1).

[6] Lee N Y, Chung K S, Jin J S, et al. The Inhibitory Effect of Nodakenin on Mast-Cell-Mediated Allergic Inflammation Via Downregulation of NF-κB and Caspase-1 Activation[J]. Journal of Cellular Biochemistry, 2017, 118(11): 3993-4001.