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Discussion on the Molecular Mechanism of Drynaria Total Flavonoids in Treating Hormonal Femoral Head Necrosis Based on Network Pharmacology

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ABSTRACT. *Objective: To explore the molecular mechanism of total flavonoids of Rhizoma Drynariae in the treatment of steroid-induced femoral head necrosis based on network pharmacology. Methods: The active compounds of total flavonoids from Rhizoma Drynariae and their targets were obtained by using the database of pharmacological component analysis platform of traditional Chinese medicine system. On this basis, three topological structure eigenvalues including node connectivity, node compactness and node betweenness were calculated. The network of "drynaria total flavonoids-active compounds-potential targets" was constructed by Cytoscape, and the protein interaction network was constructed by String database and Cytoscape. Results: determination of blood calcium and blood phosphorus contents: the differences between the experimental group and the model group were statistically significant (P<0.05). The target CYP19A1 is connected to the component 6 times, which is of great significance to the treatment of steroid-induced femoral head necrosis with drynaria flavonoids. Based on the gene ontology database GO and KEGG pathway database, the molecular function, intracellular localization and biological reactions and pathways involved in the protein encoded by the drug target gene are determined. GO analysis results show that the biological pathways of the intersection targets mainly include chemical reactions, steroid metabolic processes, cell responses to chemical stimuli, etc. Conclusion: Total flavonoids of Rhizoma Drynariae can regulate the content of blood calcium and blood phosphorus, which is helpful for the formation or calcification of new bone. Total flavonoids of Rhizoma Drynariae have the characteristics of multi-component and multi-target in the treatment of femoral head necrosis. The mechanism of Rhizoma Drynariae total flavonoids in the treatment of steroid-induced femoral head necrosis is multi-target and multi-system. Besides influencing bone metabolism related pathways, it can also affect a variety of metabolic pathways in vivo.*

KEYWORDS: *Total flavonoids from Rhizoma Drynariae, Hormonal necrosis of femoral head, Action mechanism, Access, Targets, network pharmacology*

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1. Introduction

Osteocrosis of the femoral head (ONFH) is a pathological process of osteocytic ischemia, degeneration, necrosis, trabecular bone rupture, and femoral head collapse caused by various causes of blood circulation disorder of the femoral head. One of the diseases can be divided into two major categories of traumatic and non-traumatic clinically [1]. Studies have shown that various cytokines play an important role in the occurrence and development of steroid-induced femoral head necrosis. With the gradual acceleration of population aging, steroid-induced femoral head necrosis has become an important public health problem facing the world. By 2020, there will be about 12.3 million patients with steroid-induced femoral head necrosis in the United States over 50 years old [2]. At present, the clinical treatment of steroid-induced femoral head necrosis tends to be diversified, but western medicines such as calcitonin, bisphosphate and fluoride are still the main ones. However, these drugs can only improve patients' clinical symptoms and delay their illness, and cannot fundamentally improve bone metabolism, nor can they restore the dynamic balance between osteogenesis and osteoclast process, and have certain side effects [3]. The traditional Chinese medicine treatment of ONFH takes "tonifying kidney and strengthening bone" as the treatment principle, takes "kidney governing bone and marrow" as the formulation principle, and drynaria total flavonoids as the formulation main drug. Studies have shown that total flavonoids of Rhizoma Drynariae have strong anti-osteonecrosis effect on knee hormone-induced femoral head. Experiments have shown that total flavonoids of Rhizoma Drynariae reduce cartilage lesions and significantly reduce Mankin cartilage integral. In this study, we screened and predicted the relevant targets of drynaria total flavonoids and ONFH, and analyzed them to explore the possible mechanism of drynaria total flavonoids in the treatment of ONFH.

2. Materials and Methods

2.1 Data

Through searching the pharmacological database and analysis platform (TCMSP) of traditional Chinese medicine system, all component data of total flavonoids in Rhizoma Drynariae were mined and screened according to the component toxicity pharmacokinetic (ADME) parameters [4]. Its main functions include target prediction of traditional Chinese medicine components, functional analysis of targets, visualization of related networks and comparative analysis of traditional Chinese medicines, and establishment of chemical composition database of total flavonoids in Rhizoma Drynariae.

2.2 Target network construction and analysis

TCMSP target is used to predict the target of the above-mentioned blood-entering active ingredients, and network imaging software Cytoscape3.2.1 is used to

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construct relevant networks for the active ingredients and the target of action [5]. Most Chinese herbal medicines contain as many as 50 or even thousands of compounds, but only a few compounds show ADME characteristics with potential biological effects. Therefore, it is urgent to evaluate the effects and risks of Chinese herbal medicine components on human body. The cell solution was added to 96-well enzyme plate as enzyme source to measure ALP activity. Slow down of the conversion rate of vitamin D into active vitamin D is the direct cause of steroid-induced femoral head necrosis. The interaction network of biomolecules is visually displayed, and the nodes of the network are reasonably distributed through a force-oriented algorithm to obtain a clear visual framework.

2.3 Screening of key genes and prediction of therapeutic effect on steroid-induced femoral head necrosis

Key genes refer to genes that play an important role in biological processes and can regulate other genes to play a role in related pathways [6]. The network diagram of direct and indirect target regulation of drynaria flavonoids for ONFH treatment can be obtained by fusing the two drawn network diagrams and extracting the intersection network by using the Cytoscape software. There is a negative metabolic correlation between iron and calcium ions. Reducing iron ions can improve calcium ions in osteoblasts, and it is speculated that iron ions have a close relationship with bone metabolism. Performing signal path enrichment analysis on the obtained key target points through a gene enrichment analysis plug-in Clue GO, and displaying the results in a graphical form in the form of nodes; Furthermore, the intersection target enrichment of drynaria fortunei total flavonoids and steroid-induced femoral head necrosis disease was found to be the most significant ($P < 0.05$) from numerous related annotations.

2.4 Constructing protein interaction network

TCMIP is based on the gene ontology database GO and KEGG pathway database to determine the molecular function, intracellular localization and biological reactions and pathways involved in the protein encoded by the drug target gene. The potential target of drynaria fortunei total flavonoids for the treatment of steroid-induced femoral head necrosis is introduced into String, the species is selected as "human", protein interaction information is obtained, and note1, note2 and combination score information are introduced into Cytoscape to draw protein interaction network. The KEGG database can screen out significantly enriched pathways by inputting target targets. When the P value of the pathway is less than 0.05, it is considered to be a significant key pathway. The smaller the P value, the higher its significance.

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3. Result

3.1 Effect of drynaria total flavonoids on serum Ca and p contents

The comparison of blood calcium between the two groups was tested by single factor ANOVA, and the difference was statistically significant (P <0.05); the blood calcium content of the experimental group and the model group was significantly different (P <0.05), It is suggested that the calcium content of the model group is significantly lower than that of the experimental group; it is speculated that the specific mechanism may be the expression level of iron transporter-related proteins through the withered node, thereby regulating the absorption of iron in the intestine. Total flavonoids of Rhizoma Drynariae can obviously increase the elastic strain, maximum stress and elastic modulus, which indicates that total flavonoids of Rhizoma Drynariae can improve the geometric shape and internal material properties of bone to some extent. Studies have shown that [7] Rhizoma Drynariae total flavone extract can induce osteoblast differentiation, promote bone anabolism, prevent bone mass reduction and deterioration of trabecular microstructure, and can be clinically used for treating metabolic bone diseases such as steroid-induced femoral head necrosis. Table 1 below shows the comparison of blood phosphorus and calcium levels between the two groups ($\overline{x}$ ±s).

Table 1 Comparison of blood phosphorus and calcium levels between the two groups ($\overline{x}$ ±s)

Group	Number of experiments	Blood calcium (mmol/L)	Blood phosphorus (mmol/L)
Model group	7	2.403±0.122	1..332±0.052
Experimental group	7	2.642±0.476	1.216±0.031

3.2 Key gene analysis results

According to MCC algorithm, the top 10 targets with significant degree are obtained as the key genes in the intersection targets of drynaria total flavonoids and steroid-induced femoral head necrosis diseases. PTH can finely regulate bone synthesis and catabolism, and plays an important role in differentiation, maturation and apoptosis of osteoblasts and osteoclasts. The cartilage surface of femoral head is rough, and some chondrocytes are clustered and hypertrophy. Bone trabeculae in subchondral and femoral head are atrophic, sparse, structurally disordered and fractured. Some osteocyte necrosis can be seen in bone trabeculae. The color of key gene nodes from light to dark and the size of nodes from small to large all indicate that the importance of nodes in the network increases. See Table 2 for the pathways that enrich the number of key genes ≥3.

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Table 2 Pathways enriched with ≥3 key genes

Path	Enrich key genes	P
Osteorlast differentiation	8	1.03×10^{-12}
Estrogen signaling pathway	5	5.71×10^{-10}
Pcolactin signaling pathway	6	2.63×10^{-9}
Oxytocin signaling pathway	9	7.17×10^{-11}

3.3 Analysis of protein interaction network

In this study, 7 compounds of total flavonoids from Rhizoma Drynariae were retrieved through BATMAN-TCM database, and their targets were further retrieved through BATMAN-TCM combined with GeneCards database, and then 6 online databases such as recognized TTD were retrieved to obtain steroid-induced femoral head necrosis disease targets. Naringin can increase bone mineral density of model animals, promote limb blood circulation to remove blood stasis and further promote fracture healing, and its effect is dose dependent. It is believed that it plays an important role in the anti-steroid femoral head necrosis of drynaria total flavonoids, in which the degree value of target ESR1 is more than 2 times of the median, which may be the key target for the anti-steroid femoral head necrosis of drynaria total flavonoids. Topological structure analysis results (Table 3).

Table 3 Analysis results of topological structure of protein interaction network

Serial number	Potential targets	Node connectivity
1	NR3C1	12
2	ESR2	8
3	UGT2B17	3
4	ALOX12	6

4. Discussion

Under normal circumstances, bone resorption and bone formation maintain balance through coupling mechanism, and this effect is accomplished through the action of osteoclasts and osteoblasts. The long-term use of anti-steroid femoral head necrosis drugs, especially the gastrointestinal reactions, musculoskeletal, mandibular necrosis and other adverse reactions caused by bisphosphonates, has gradually been paid attention [8], and new drugs for the treatment of steroid femoral head necrosis are still being continuously explored and developed. Predicting the effective components and potential targets of Rhizoma Drynariae total flavonoids in the treatment of steroid-induced femoral head necrosis based on integrated pharmacological methods is helpful to adapt to and reflect the characteristics of multi-components, multi-targets and synergistic effects of traditional Chinese medicine. These passages are relatively complicated and include a plurality of

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downstream passages. The results of this study show that the mechanism of total flavonoids of Rhizoma Drynariae acting on ONFH is mainly concentrated in PI3K/Akt, HIF-1, Notch, DNA replication, apoptosis and other signal pathways. Inhibit the synthesis of matrix macromolecules such as collagen II and proteoglycan polymers; At the same time, it can stimulate Ming-degrading enzyme and promote synthesis and secretion of prostaglandin (PGE2), thus causing cartilage destruction and bone absorption. Only by its continuous division and proliferation can it produce abundant collagen matrix and form more bone through mineralization. Promoting bone tissue formation by stimulating osteoblast proliferation is one of the main methods to treat steroid-induced femoral head necrosis.

In this study, 14 active components, 26 potential targets and 51 correlations of total flavonoids from Rhizoma Drynariae were screened, involving 3 major pathways and 5 major biological processes. Based on the application of big data and model calculation, an intelligent and networked platform is constructed to analyze the correlation between natural monomer components of various Chinese medicines and disease-related targets and pathways. TNF mainly includes two receptors, TNFR1 and TNFR2. TNFR1 signaling pathway can activate and induce the activation of many genes and is mainly controlled by two different pathways. P53 is recognized as a protein molecule related to cell apoptosis and has the function of inducing cell apoptosis. P53 acts as a checkpoint in G1 phase and can decide whether to start the apoptosis process. Alcohol extract of total flavonoids from Rhizoma Drynariae can increase the activity of ALP in cells and obviously promote the synthesis and secretion of osteocalcin in cells. However, due to the weight-bearing effect of the femoral head and the anatomical characteristics of its internal vessels, local vessels are prone to fat embolism, which causes local microcirculation disturbance and leads to avascular necrosis of femoral head tissue. Total flavonoids can improve the level of BMP-2 and participate in bone reconstruction. Several experimental studies have proved that total flavonoids can reduce bone turnover rate and increase bone density.

Bone marrow stromal cells (BMS) are pluripotent stem cells that can differentiate into not only osteoblasts, but also adipocytes, chondroblasts, myoblasts and even nerve cells. Oxygen inducible factor 1α(HIF-1α) is the most characteristic hypoxia response signal pathway in organisms. Another example is the AGE/RAGE signaling pathway, which can regulate cell and system responses by regulating protein kinase C(PKC) and p38 MAPK signaling pathways, thus increasing the expression of bone matrix protein. Previous studies have shown that traditional Chinese medicine compound preparation can inhibit osteoclasts by regulating the expression of osteoclast differentiation regulatory factors OPG, RANKL, etc. Other studies [9] have shown that naringenin can improve the expression of vascular endothelial factor and VEGFR-2 and promote the formation of bone vessels. Total flavonoids of Rhizoma Drynariae can improve ALP activity in UMR-106 cells, and have corresponding changes at different times, with certain dose-effect and time-effect relationship, with 48h being the most ideal. PGE2 can induce inflammation, promote local vasodilation, and increase capillary permeability, white blood cell exudation, etc., leading to inflammatory response. Liu Tingting et al [10] believe

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that lipid metabolism imbalance plays a key role in the early onset of femoral head necrosis and is one of the important factors causing femoral head necrosis. The enrichment analysis results of total flavonoids in Rhizoma Drynariae showed that the network cluster also covered EB virus infection, ubiquitin-mediated proteolysis, ethanol poisoning, cancer and other signal pathways.

Through the screening and analysis of key genes, it can be seen from Table 3 that there are 4 enrichment numbers of key genes in these pathways, and their P values are not more than 1.96× 10-9, further illustrating the importance of these pathways. From the point of view of single drug, the kidney-tonifying and yang-boosting drug Rhizoma Drynariae total flavonoids and its active substance Rhizoma Drynariae total flavonoids have good clinical efficacy and research foundation, and have the function of promoting osteoblast differentiation and proliferation. Naringenin activates ER α phosphorylation in UMR 106 osteoblast-like cells through estrogen receptor (ER)-dependent pathway, thus exerting osteogenesis. The 3H-TdR incorporation experiment shows that drynaria total flavonoids can promote the differentiation and proliferation of osteoblasts. The high dose group obviously increases the differentiation and proliferation of osteoblasts, and the longer the time, the greater the effect. Vascular embolism is more likely to cause vascular endothelial cell necrosis and arteriolar spasm in femoral head in some idiopathic diseases. Although artificial joint replacement surgery is feasible for advanced steroid-induced femoral head necrosis, due to certain short-term and long-term complications of joint replacement surgery, such as loosening and abrasion of components and osteolysis, these complications cannot be completely solved at present and are expensive.

5. Summary

Oral administration of total flavonoids of Rhizoma Drynariae, an effective component of traditional Chinese medicine, can improve the symptoms of osteoarthritis in patients with steroid-induced femoral head necrosis. A large amount of hormones can significantly reduce the calcium content in blood and significantly increase the blood phosphorus content. Total flavonoids of Rhizoma Drynariae can effectively prevent hormone-induced calcium and phosphorus metabolic disorders and promote calcium salt deposition and bone formation. The main active components in total flavonoids of Rhizoma Drynariae can exert anti-osteoporosis effect on multiple targets and multiple pathways. The mechanism of Rhizoma Drynariae total flavonoids in preventing and treating primary sex hormone-induced femoral head necrosis may be through promoting osteoblast activity and growth, stimulating osteoblast proliferation and promoting bone tissue formation. MAPKs plays an important role in the development of osteoblasts and regulates bone formation by acting on osteoblasts. According to the different conditions of patients and diseases, individualized multi-target and multi-system therapy should be applied to local key pathways according to individual conditions.

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References

[1] Zhou Jinliang, Ruan Zhilei. Experimental study of the effect of flavonoids of Gusuibu on hormonal femoral head necrosis [J]. Health Frontier, Vol. 23 (2016) No. 5, p. 147-147.

[2] Zeng Hui, Zhao Xubing, Cong Cong, et al. Effect of flavonoids of Bupleurum on expression of interleukin-6 and osteocalcin in periodontal tissue of rats with periodontitis [J]. Journal of Shanxi Medical University, Vol. 46 (2015) No. 4, p. 324-327.

[3] Shi Xiaozhi [1], Zhao Yongfang [2], Guo Hailing [2]. Effects of flavonoids and icariin on the proliferation and protein expression of human bone marrow stromal stem cells [J]. Chinese Medical Journal, (2015) No. 5, p. 693-695.

[4] Zeng Zhikui, Huang Feng, Li Yue, et al. The effect of flavonoids on the expression of angiogenesis factor induced by Masquelet in rats [J]. Chinese Journal of Traditional Chinese Medicine, (2019) No. 10, p. 2345-2348.

[5] Zhang Chunqi, Liu Penghe. Clinical Observation on 68 Cases of Postmenopausal Female Osteoporosis Treated with Bone Flavonoids [J]. Clinical Research of Traditional Chinese Medicine, Vol. 7 (2015) No. 28, p. 109-110.

[6] Li Yang, Kang Qian, Rong Chan, et al. Study on the effect of total flavonoids of Gushenbu on the proliferation, differentiation, mineralization and apoptosis of MLO-Y4 cells [J]. Chinese Journal of Osteoporosis, (2015) No. 5, p. 592-598.

[7] Sun Zongpi, Xu Chan, Wang Juan. Observation of curative effect of osteoclasts combined with total flavonoids of osteoclasts and alfacalcidol [J]. Journal of Chronic Diseases, (2016) No. 3, p. 327-329.

[8] Cai Qunbin, Li Ding, Li Yue, et al. The effect of total flavonoids of Bupleurum on Masquelet technique-induced intravascular membrane formation [J]. Journal of Guangzhou University of Traditional Chinese Medicine, Vol. 34 (2017) No. 6, p. 867-871.

[9] Qiao Weiping, Li Huiying, Wang Lihe. Bushen Huoxue Decoction combined with intermediate frequency pulsed electrotherapy for the treatment of 16 cases of early ischemic necrosis of femoral head [J]. Journal of Traditional Chinese Medicine, Vol. 28 (2015) No. 1, p. 13-14.

[10] Liu Tingting, Gu Ting, Ge Yanan, et al. Enrichment process of total flavonoids of Gusuibu and its protective effect on Aβ (25-35) -induced PC12 injury cells [J]. Science and Technology Innovation, (2017) No. 3, p. 166-167.