Study on compound Duzhong Jiangu granule in the treatment of knee osteoarthritis based on transcriptome analysis strategy

Si-Cong Li1, Jun-Bo Zou2, Wu-Lin Kang1, Pu-Wei Yuan3

1The First Clinical College of Medicine, Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang 712000, China. 2College of Pharmacy, Shaanxi University of Chinese Medicine, Xianyang 712000, China.

2These authors contributed equally to this work and are co-first authors for this paper.

*Correspondence to: Wu-Lin Kang, Pu-Wei Yuan. The First Clinical College of Medicine, Affiliated Hospital of Shaanxi University of Chinese Medicine, Vice No.2 Weiyang West Road, Xianyang 712000, China. E-mail: 382019202@qq.com; spine_surgeon@163.com.

Abstract

Background: Based on the whole animal model, to study the efficacy of Compound Duzhong Jiangu Granule in the treatment of primary osteoarthritis (POA) model of postmenopausal kidney deficiency type in Hartley female guinea pigs after ovarioectomy, and the correlation between gene expression of bone marrow tissue, cartilage tissue, and knee osteoarthritis. Methods: 38 3-months-old Hartley female guinea pigs after one week of adaptive feeding were weighed about 400 g ± 20 g, numbered, and sorted by ear tag. Six of them were selected as normal groups by looking up random number tables. The remainder were removed from the bilateral ovaries to construct the postmenopausal kidney deficiency model, and castrated guinea pigs were used to construct the postmenopausal kidney deficiency POA model. After the modeling cycle, a guinea pig from the blank group and a guinea pig from the model group were sacrificed and the right knee was observed. The model was established and the experiments continued. There were five guinea pigs in the blank group, and the remaining model guinea pigs were randomly divided into model control group, high-dose group of compound Duzhong Jiangu granules, middle-dose group of compound Duzhong Jiangu granules, low-dose group of compound Duzhong Jiangu granules and design group, with 5 guinea pigs in each group. Blanks and model groups were given a cellulose sodium solution by gavage. The guinea pigs were sacrificed after 30 days of intrastratic administration. The left knee cartilage and bone marrow of the blank group, model group, middle dose group, and high dose group of compound Duzhong Jiangu granule were collected and applied to transcriptome sequencing, and the sequencing data were analyzed, including differential gene expression analysis, functional enrichment analysis of database established by Gene Ontology federation (GO) and Kyoto Encyclopedia of Gene and Genome (KEGG) pathway enrichment analysis. The complete specimens of the right knee joint were collected, and the morphological changes of the cartilage of the right knee joint in each group were observed by saffron rapid green staining, and the subchondral bone was quantitatively analyzed by Micro CT so that the expression of TRAP6, MIP-1p and IL-1p protein in NF-kappa B signaling pathway was detected by Western Blot technique (WB). Results: The results of Safranin Fast Green staining showed that Compound Duzhong Jiangu Granules could effectively reduce the degree of morphological damage of articular cartilage in guinea pigs with the POA model. According to the analysis results of the subchondral bone structure under Micro CT, Compound Duzhong Jiangu Granules can improve the bone condition of the POA model, thus delaying the process of degenerative changes of the knee joint. From the results of transcriptome analysis, Compound Duzhong Jiangu Granules can effectively improve knee osteoarthritis. Conclusions: The effect of Compound Duzhong Jiangu Granules on OA is obvious, and its mechanism may be related to the expression of genes GZMK, Jchain, Igk, IGHV3-74, IGHV3-11, IGHV4-1, CCL5, and IGRV1–39.

Keywords: knee osteoarthritis; transcriptome sequencing; compound Duzhong Jiangu granules; micro-CT
Introduction

Knee osteoarthritis is a common cause of joint function loss and physical disability caused by chronic pain. Bone marrow edema is often accompanied in the pathogenesis of knee osteoarthritis. Studying cartilage tissue and bone marrow tissue is of great help to the understanding of knee osteoarthritis. Compound Duzhong Jiangu Granules provides a new idea for the treatment of knee osteoarthritis. Through a large number of clinical studies, it has been found that Compound Duzhong Jiangu Granules can effectively relieve pain in patients with knee osteoarthritis. Through a large number of clinical studies, it has been found that Compound Duzhong Jiangu Granules can effectively relieve pain in patients with knee osteoarthritis.

Knee osteoarthritis (KOAK) is a degenerative disease, which is common in middle-aged and elderly people [1]. Knee osteoarthritis is a common cause of joint function loss and physical disability caused by chronic pain. With the increase in the age of the general population, the incidence of knee osteoarthritis is also increasing. Therefore, knee osteoarthritis has become a major health problem worldwide [2]. According to data released by the World Health Organization in February 2021, about 343 million people worldwide have osteoarthritis [3]. By 2030, osteoarthritis will become the main cause of disability in the elderly. The main pathological features of osteoarthritis are cartilage and bone remodeling. With the development of the disease, significant pathological changes occur in subchondral bone tissue, synovial tissue, and surrounding joint ligaments and muscle tissue [4]. At present, the treatment of osteoarthritis is relatively simple. The clinical use of non-steroidal anti-inflammatory drugs alone is not effective, and it is easy to cause gastrointestinal discomfort. Therefore, although non-steroidal anti-inflammatory drugs can temporarily reduce or control the patient's condition, they will have relatively high risk factors and should not be used for a long time. Traditional Chinese medicine has certain unique advantages in the treatment of osteoarthritis [5].

Compound Duzhong Jiangu Granule is composed of 12 kinds of traditional Chinese medicine, such as Duzhong, Paronyia Radix Alba, Dipsaci Radix, Radix Astragali, Fructus lycii, Radix Achyranthis Bidentatae, Notoginseng Radix et Rhizoma, Spatholobi caulis, Ginseng Radix et Rhizoma, Angelicae Sinensis Radix, Phellodendri chinensis Cortex and Clematidis Radix et Rhizome [6]. It has the effects of nourishing the liver and kidney, nourishing blood and invigorating tendons, dredging collaterals, and relieving pain [7]. It is mainly used for the treatment of swelling, pain, and dysfunction caused by knee OA. The 12 herbs in the prescription can act on the target 5-LOX and COX-2. The 9 herbs except Notoginseng Radix et Rhizoma, Clematidis Radix et Rhizome, and Spatholobi caulis can act on the target LTα/H. The compatibility of each herb can act on the target 5-LOX, COX-2, and LTα/H at the same time, indicating that Compound Duzhong Jiangu Granules have the characteristics of multi-component, multi-channel and multi-target synergistic treatment of osteoarthritis [8].

RNA sequencing (RNA-seq) is a new sequencing technology developed in 2008. It refers to the extraction of total RNA from tissues or cells by bioinformatics analysis methods and converts it into a cDNA fragment library. RNA is sequenced by high-throughput sequencing technology. This technology can quickly and comprehensively obtain all transcripts of tissues or cells of specific species to reveal the level and changes of gene expression and further clarify the mechanism of its specific molecules [9].

As the primary clinical manifestation of knee osteoarthritis, pain can be derived from joint bone lesions or periarticular soft tissue lesions. At present, there is no unified conclusion on the research results of pain factors in knee osteoarthritis. Cartilage injury, subchondral bone lesions, synovitis, meniscus tear, ligament injury, joint-free body, intra-articular cyst, and other factors are the main factors causing knee osteoarthritis pain, swelling, and limited activity. In recent years, bone marrow edema has attracted the attention of the majority of clinical workers because of its important role in the pain caused by knee osteoarthritis. Through this study, it was found that bone marrow edema was moderately correlated with joint pain in patients with knee osteoarthritis [10]. (WOMRS score and WOMAC pain index in patients with knee osteoarthritis $r = 0.752 > 0.5$, and joint pain VAS $r = 0.650 > 0.5$, $P < 0.001$).

Therefore, this experiment analyzed bone marrow tissue and cartilage tissue to further study knee osteoarthritis. In this study, the osteoarthritis model of guinea pigs was induced by removing the ovaries of female guinea pigs, and the intervention treatment of Compound Duzhong Jiangu Granules was carried out by intragastric administration. The therapeutic effect of Compound Duzhong Jiangu Granules on guinea pigs was analyzed, and the gene changes of cartilage tissue and bone marrow tissue were explored by transcriptome sequencing, which provided theoretical and experimental basis for the treatment of osteoarthritis with Compound Duzhong Jiangu Granules.

Materials

Animal

Thirty-eight 3-month-old female Hartley guinea pigs (provided by Chengdu Dashuo Experimental Animal Co., Ltd., license number: SCXK (Sichuan) 2020-030), weighing 400 ± 20 g, temperature (22 ± 2) °C, relative humidity 50%–60%, adaptive feeding for 1 week, 5 in 1 cage. Feed in the animal laboratory of the College of Pharmacy, Shaanxi University of Traditional Chinese Medicine. The experimental process was carried out following the 'Laboratory Animal Welfare Ethics Review Technical Specification' and approved by the Laboratory Animal Ethics Committee of Shaanxi University of Traditional Chinese Medicine (No. SUCMDL2022104002).

Drugs and reagents

Drugs. Compound Duzhong Jiangu Granules (Beijing Shuanghe Co., Ltd., 14000139421), Diclofenac Capsules (TRB Pharmaceutical Co., Ltd., 02552), CMC-Na (Tianjin Kemioi Chemical Reagent Co., Ltd., 20190709), anhydrous ethanol (100092683), xylene (10023418), neutral gum (10004160) are purchased from Sinopharm Chemical Reagent Co., Ltd.; the Safranin Solid Green Bone Tissue Dyeing Solution Kit (G1053) and Differentiation Solution (G1040) were purchased from Service.

Reagents. 0.5% CMC-Na: 5 g of CMC-Na was weighed and boiled in 800 mL of water, then a small amount of it was sprinkled on the boiling water surface many times, stirred until dissolved, constant volume to 1000 mL, and stored at room temperature.

Apparatus


Method

Animal grouping, modeling, and administration

The living conditions of all experimental guinea pigs were controlled at a temperature of (23 ± 2) °C, relative humidity of (55 ± 5) %,
daily light for 12 hours, and free access to water and food. Feeding in the animal laboratory of the College of Pharmacy, Shaanxi University of Traditional Chinese Medicine, all animal experiments were in line with the requirements of the Animal Ethics Committee of Shaanxi University of Traditional Chinese Medicine.

After one week of adaptive feeding, 38 guinea pigs were divided into 6 groups by random number table method: blank control group (Blank Control), model group (Model), positive drug group (Positive drug), and different dose groups of Compound Du Zhong Jiangus Granules (FFDZ) [11]. In addition to the blank group of guinea pigs, the rest of the guinea pigs established the POA model. After fasting for 2 days and water deprivation for 1 day before the operation, the remaining groups of guinea pigs were anesthetized by a small animal anesthesia machine. After about 5 mins, the limbs were soft. The prone position was used to repeatedly touch the rib ridge angle of the guinea pig with the hand, and the outer and lower sides were centered at about one horizontal finger. In the radius of 3 cm, the surface coarse hair was first cut with large scissors, and then the deep fine soft hair was carefully removed with small scissors, without injuring the skin. Then, iodophor was used to sterilize 3 times repeatedly, and the surgical towel was spread. The surgeon wore gloves and disinfected again. A longitudinal slightly lateral incision was made at about 0.8 cm below the rib ridge angle and about 1 cm outside through the cortex. About 1 cm, the skin, subcutaneous tissue, and fascia were incised in turn, and the posas major and posterior peritoneum were cut open with tissue scissors. Light gray ascites flowed out, and the surrounding adipose tissue was slowly pulled with small tweezers. Oval ovaries and pink fallopian tubes of small soybean size were visible. The fallopian tubes and surrounding blood vessels were carefully peeled and ligated. After the ovaries were removed, the adipose tissue was returned. The posas major, subcutaneous fascia, and skin were sutured in turn, and the abdominal cavity was closed. Disinfection of incision with iodophor.

After the establishment of the model, the positive drug group was given 9 mg/kg diacerein capsule aqueous solution by gavage; the clinical dosage of Compound Du Zhong Jiangus Granules was 12 g × 3 packs/day. The dosage of guinea pigs was calculated according to the body surface coefficient method. Therefore, 3.24 g/kg, 6.48 g/kg, and 12.96 g/kg doses of Compound Du Zhong Jiangus Granules were selected to intervene in the Compound Du Zhong Jiangus Granules group. The rats in the blank group and the model group were given the same volume of 0.5 % CMC-Na solution once a day for 30 days.

**Specimen collection and processing**

**Figure 1** Cartilage and bone marrow of guinea pig knee joint. (A) Cartilage and bone marrow. (B) Saffron solid green staining results of guinea pig knee joint. (C) MICRO-CT scanning of guinea pig knee joint. (D) Analysis of MICRO-CT scan results.

**Collection and processing of knee joint.** According to the protein imprinting analysis method reported in the literature.

**Collection and processing of bone marrow and cartilage.** The skin was prepared around the left knee joint. The large scissors quickly removed the surrounding muscles and other tissues. The small tissue scissors opened the articular cavity, cut the articular cartilage with a scalpel, cut the tibial plateau with small scissors, and stripped the bone marrow in the tibia with small tweezers, as shown in Figure 1A. The cartilage and bone marrow were taken and placed in a 1.5 ml EP tube and stored in an –80-degree refrigerator.

**Transcriptome sequencing, screening of differentially expressed genes, and correlation analysis**

**Transcriptome sequencing of cartilage and bone marrow mRNA.** The articular cartilage tissue and bone marrow tissue of each of 3 guinea pigs were randomly mixed as a sequencing sample in the four groups of Control, Model, FFDZ (high dose group), and FFDZ (medium dose group), and the total RNA was extracted from the articular cartilage tissue and bone marrow tissue of each sample by Trizol lysis solution.

Eukaryotic mRNA with polyA structure was enriched by magnetic beads containing Oligo (dT), and mRNA was fragmented by ion disruption. Then cDNA was synthesized by reverse transcription, purified, ligated at the end, and amplified by PCR to establish a library. After the library was qualified, double-end sequencing was performed based on the Illumina sequencing platform. Library construction and sequencing were completed by Guangzhou Gideo Biotechnology Co., Ltd. The original sequence (raw reads, RR) obtained after sequencing was filtered to remove some low-quality sequences with adapters to obtain high-quality clean reads (clean reads, CR), and then the filtered CR was compared to the reference genome using HISAT2 software. HTSeq was used to compare the Read Count value of each gene as the original expression level of the gene, and FPKM (Fragments Per Kilobases per Million fragments) was used to standardize the expression level, and then DESeq was used to analyze the difference of gene expression. The conditions for screening differentially expressed genes were: expression difference log2 (fold change, FC) > 1, significant P < 0.05. After obtaining the differential genes, the Gene Ontology (GO) database established by the Gene Ontology Consortium and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were compared, and then the differential gene significance enrichment analysis was performed by the hypergeometric distribution method.
Correlation analysis. Based on the ggplot2 and ggcorrplot R language packages, the correlation analysis of bone marrow and cartilage mRNA was carried out, and the intersection analysis of bone marrow and cartilage mRNA with a correlation absolute value greater than 0.4 was carried out.

Western blot analysis. According to the protein imprinting analysis method reported in the literature, the main steps: using the coomassie brilliant blue method for the determination of total protein concentration [12]. Then, the total protein samples were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and transferred to the nitrocellulose (NC) membrane. Then, the NC membrane was incubated with the following primary antibodies overnight at 4 °C: (A) polyclonal rabbit anti-IL-1β (1:1000); (B) polyclonal mouse anti-MIP-1β (1:1000); (C) polyclonal rabbit anti-TRA6 (1:1000); polyclonal mice anti-GAPDH (1:10000). Finally, the NC membrane was washed with TBSB and incubated with the corresponding horseradish peroxidase (HRP)-bound secondary antibody. The NC film was visualized using substrate luminescent reagents and image analysis was performed.

Statistical analysis. SPSS 26 software was used to analyze the experimental data. The results were expressed as mean ± standard deviation (x ± s). One-way analysis of variance was used to analyze the differences between groups. P < 0.05 indicated that the difference was statistically significant.

Results

Compound Duzhong Jiangu granules can slow down the progress of knee osteoarthritis

In the control group, the joint structure of the guinea pigs was complete the surface was smooth, and the matrix staining was uniform. After staining, the cartilage color distribution was obvious, the chondrocytes were arranged regularly, and the bone tissue was compared with the cartilage tissue. In the Model group, the joint structure of guinea pigs was destroyed, some of them had cartilage defects, cartilage staining was lost, cartilage matrix was lost, there was no change in color level, and the contrast between bone tissue and cartilage tissue was blurred. The joint structure of guinea pigs in each administration group of Compound Duzhong Jiangu Granules was clear, the cartilaginous safranin staining was slightly deeper, the layers were still retained, the cells were arranged slightly neatly, and the bone tissue and cartilage tissue were more obvious, as shown in Figure 1B. Therefore, it can be clearly seen that Compound Duzhong Jiangu Granules can effectively reduce the progress of knee osteoarthritis.

Under micro-CT observation, compound Duzhong Jiangu granules can effectively treat knee osteoarthritis

Micro-CT subchondral bone imaging of guinea pig knee joint specimens can be found: the subchondral bone trabeculae of the Control group are uniform, regular in shape, and arranged in a more orderly manner; compared with the Control group, the number of subchondral bone trabeculae in the Model group was significantly reduced, the arrangement of bone trabeculae was disordered and uneven, the distribution was sparse and the thickness was thinner; the subchondral bone imaging of the Positive Drug group was similar to that of the Control group; compared with the Model group, the number of bone trabeculae in the FFDZ (low) group increased, and the number of bone trabeculae in the FFDZ (middle) and FFDZ (high) groups increased significantly, arranged neatly, distributed closely and regularly, and the thickness was also significantly thickened, as shown in Figure 1C.

The results of micro-CT subchondral bone microstructure morphological parameters and structural parameters of guinea pig knee joint specimens: Compared with the Control group, the bone volume fraction (BV/TV), trabecular thickness (Tb. Th), trabecular number (Tb. N), and bone surface area tissue volume ratio (BS/TV) in the Model group were significantly decreased (P < 0.05). The ratio of bone surface area to bone volume (BS/BV), trabecular separation (Tb. Sp), structural pattern index (SMI), and trabecular pattern factor (Tb. Pf) were significantly increased (P < 0.01). Compared with the Model group, the bone volume fraction (BV/TV), trabecular thickness (Tb. Th), and trabecular number (Tb. N) in the FFDZ (middle) and FFDZ (high) groups were significantly increased (P < 0.05), the trabecular separation (Tb. Sp) (P < 0.05), and the ratio of bone surface area to bone volume (BS/BV) in the FFDZ (high) group was significantly decreased (P < 0.01). The results are shown in Figure 1D.

Transcription sequencing results

The results of sequencing quality distribution were shown in Figure 2A, and [log2FC] ≥ 1 and P < 0.05 were used as screening conditions to screen mRNAs with significant differences. By finding the intersection gene of the blank group and the model group, as shown in Figure 2B, the mRNA of the difference between the bone marrow group and the cartilage group was displayed by the difference in expression, as shown in Figure 2C. It can be seen from the figure that there are great differences between the blank group and the model group in both the bone marrow group and the cartilage group. There are also significant differences between the model group and the compound Duzhong Jiangu granule group, so transcriptome sequencing can be used for subsequent analysis.

Figure 2 Cartilage and bone marrow gene sequencing. (A) Bone marrow and cartilage gene sequencing scatter plot. (B) Intersection gene Wayne diagram. (C) Intersection gene expression heat map. (D) The up-regulated and down-regulated map of differential gene expression. Grouping information: Bone marrow (A, blank group; B, model group; C, FFDZ middle; D, FFDZ high); Cartilage (E, blank group; F, model group; G, FFDZ middle; H, FFDZ high).
The bone marrow group and the cartilage group were analyzed separately by volcano map as shown in Figure 2D. In the bone marrow group, 182 genes were up-regulated and 68 genes were down-regulated compared with the model group. In the cartilage group, there were 1424 up-regulated genes and 469 down-regulated genes in the blank group compared with the model group. The intersection of the bone marrow group and the cartilage group was made into a Venn diagram as shown in Figure 3A, and 153 intersection genes were obtained.

The genes with an fpkm value greater than 10 in the intersection genes were selected to make a heat map as shown in Figure 4. It can be seen from the figure that there were differences in the expression levels between the bone marrow group and the cartilage group, the blank group, and the model group. The occurrence of knee osteoarthritis is related to the overexpression of these genes, and the high-dose group of Compound Duzhong Jiangu Granules can effectively inhibit the expression of these genes. The genes with fpkm value greater than 10 in the bone marrow group and the cartilage group were intersected to obtain GZMK, Jchain, Igk, IGHV3-74, IGKV3-11, IGKV4-1, CCL5, IGKV1-39, which were made into heat maps as shown in Figure 3B. It can be seen that the high dose of Compound Duzhong Jiangu Granules can effectively inhibit the expression of pathogenic genes.

Figure 3 Intersection gene analysis. (A) Bone marrow and cartilage intersection gene Venn diagram. (B) Genes of intersection expression heat map with fpkm > 10.

Figure 4 Gene heatmap analysis. (A) Gene expression heat map with fpkm > 10 (Bone marrow). (B) Gene expression heat map with fpkm > 10 (Cartilage).
By analyzing the intersection genes of the bone marrow cartilage group, the GO and KEGG maps as shown in Figure 5 can be obtained, and the NF-kappa B signaling pathway related to knee osteoarthritis can be selected. The related target proteins TRAF6, MIP-1β, and IL-1β were obtained in the pathways shown in Figure 6.

Effects of compound Duzhong Jiangu granules on the expression of TRAF6, MIP-1β, and IL-1β in cartilage tissue. Western blot was used to verify the protein expression levels of TRAF6, MIP-1β, and IL-1β, the core targets of KOA in Compound Duzhong Jiangu Granules. Compared with the blank group, the protein expression levels of IL-1β shown in Figure 7A, MIP-1β shown in Figure 7B, and TRAF6 shown in Figure 7C in the model group were increased (P < 0.01). Compared with the model group, the protein expression levels of IL-1β, TRAF6, and MIP-1β in the high-dose group of compound eucommia ulmoides were decreased (P < 0.01). The results echoed the transcriptome sequencing results. It can be seen that Compound Duzhong Jiangu Granules can effectively reduce pro-inflammatory factors in cartilage, thereby delaying the progress of knee osteoarthritis.

Figure 5 GO and KEGG

Figure 6 The NF-kappa B signaling pathway
Figure 7 Western blot technique experimental analysis. (A) Western Blot technique results of IL-1β. (B) Western Blot technique results of MIP-1β. (C) Western Blot technique results of TRAF6.

Discussion

Knee osteoarthritis is a slowly progressive disease, endangering the health of patients and affecting the quality of life of patients. At present, the treatment of knee osteoarthritis is mainly non-steroidal anti-inflammatory drugs. The treatment effect is not good, and it cannot effectively inhibit the progress of knee osteoarthritis. In the later stage of disease development, patients can only choose knee replacement surgery, which greatly increases the time and economic cost of patients with knee osteoarthritis. Knee osteoarthritis belongs to the category of ‘arthralgia disease, bone arthralgia’ in traditional Chinese medicine. Traditional Chinese medicine believes that the pathogenesis of bone arthralgia is the deficiency of the liver and kidney. In the process of treating knee osteoarthritis, traditional Chinese medicine focuses on the tonifying of the liver and kidney. In the study, it was found that traditional Chinese medicine has a good effect.

Compound Duzhong Jiangu granule is composed of 12 kinds of traditional Chinese medicine, such as Duzhong, Paoniae Radix Alba, Dipsaci Radix, Radix Astragali, Fructus lycii, Radix Achyranthis Bidentatae, Notoginseng Radix et Rhizoma, Spatholobi caulis, Ginseng Radix et Rhizoma, Angelicae Sinensis Radix, Phellodendri chinensis Cortex and Clematidis Radix et Rhizome [6]. In the prescription, Eucommia ulmoides have the effects of tonifying the liver and kidney and strengthening bones and muscles. Astragalus, Dipsacus asper, and Paonia lactiflora have the effects of antispasmodic and anti-inflammatory, nourishing blood and invigorating tendons, tonifying qi and solidifying the surface, softening liver and relieving pain, regulating blood vessels and immune regulation. Ginseng, Achyranthes bidentata, Angelicae sinensis, Lycii Fructus, Phellodendri Chinensis Cortex, Panax Notoginseng, and Spatholobi Caulis have the effects of relieving swelling and pain, tonifying the liver and kidney, tonifying essence and qi (Qi is the most basic substance that makes up the human body and sustains its vital activities), and strengthening bones and muscles. Clematidis Radix plays the role of dispelling rheumatism, dredging meridians, and relieving pain. Modern pharmacological studies have confirmed that Compound Duzhong Jiangu Granule can effectively improve bone and joint diseases. Its extract can directly act on chondrocytes, improve metabolism, and then maintain the normal structure and function of articular cartilage, and prevent the further development of osteoarthritis diseases [13]. Compound Duzhong Jiangu Granule is widely used in clinical practice. Liu Enxiong et al. have proved the effectiveness and safety of the Compound Duzhong Jiangu Granule in the treatment of knee osteoarthritis through clinical studies [14]. Studies have shown that Compound Duzhong Jiangu Granule is effective in the adjuvant treatment of knee osteoarthritis and can effectively reduce the subjective pain of patients. Improve the clinical symptoms of patients and have no obvious adverse reactions. Chen Zijun et al. [8] analyzed the potential pharmacodynamic substances and mechanism of action of Compound Duzhong Jiangu Granules by molecular simulation technology combined with molecular biology. It was found that Compound Duzhong Jiangu Granules acted on the LOX and COX pathways in the arachidonic acid metabolic pathway to exert the mechanism of action in the treatment of osteoarthritis. The 12 herbs in the prescription can act on the target 5-LOX and COX-2, and the 9 herbs except Notoginseng Radix et Rhizoma, Spatholobi caulis, and Clematidis Radix et Rhizome can act on the target LTA4. The compatibility of each herb can act on the target 5-LOX, COX-2, and LTA4 at the same time, indicating that Compound Duzhong Jiangu Granules have the characteristics of multi-component, multi-channel and multi-target synergistic treatment of osteoarthritis. Shu Zhan et al. through network pharmacology combined with in vitro experiments, the overall mechanism of Compound Duzhong Jiangu Granules was discussed [15]. The results showed that Compound Duzhong Jiangu Granules may regulate inflammation, chondrocyte proliferation, differentiation, apoptosis, and metabolism by acting on key targets such as TNF, STAT3, MAPK1, etc. Related pathways, play an anti-inflammatory role, regulate cell proliferation, differentiation, apoptosis, angiogenesis, and other pharmacological effects, and then play an overall effect.

In recent years, the use of transcriptomics technology to study the therapeutic target genes of diseases or the pharmacological mechanism of drugs has become a research hotspot. The use of transcriptomics to study the complex process of traditional Chinese medicine compound treatment of diseases can comprehensively
understand the effect of drugs and specific treatment mechanisms [16]. Chemokine CCLs, also known as MIP-1β, is a macrophage inflammatory protein belonging to the CC chemokine family and plays an important role in the pathogenesis of KOA. In addition to promoting osteoclast formation leading to the progression of KOA, chemokines also play an important role in other aspects of bone metabolism. CCLs can be used as a pro-inflammatory factor to measure the degree of KOA joint inflammation. It can also stimulate the synthesis of matrix metalloproteinase-3 (MMP3) in articular cartilage, which leads to the catabolism of articular cartilage [17, 18]. In the WB experiment, it was found that Compound Duzhong Jiangu Granules can effectively reduce the chemokine CCLs in cartilage, thereby treating knee osteoarthritis. RT-qPCR was performed on some core targets screened by network pharmacology. It was found that Compound Duzhong Jiangu Granules could inhibit the expression of TNF, STAT3, and MAPK1 genes in LPS-induced RAW 264.7 inflammatory cells, which was consistent with the results of PPI analysis. In patients with osteoarthritis, inflammation-related target TNF is abnormally highly expressed [19]. STAT3 is a key regulator that plays an important role in the inflammatory response [20]. Studies have found that STAT3 is the core transcription factor of bone and joint diseases, which can further lead to the occurrence of bone and joint diseases through nuclear factor kappa B (NF-κB) signaling [21]. MAPK1 is an important component of the MAPK signal transduction pathway, and the MAPK signal transduction pathway is responsible for regulating the production of pro-inflammatory cytokines, which can eventually lead to joint inflammation and destruction. Shu Zhan et al. Studies have shown that Compound Duzhong Jiangu Granules can reduce RAW 264.7 to a certain extent [15].

Studies have shown that fibroblast-like synovial cells in rheumatoid arthritis cause cartilage damage by producing inflammatory cytokines and proteases. The increase of TRAF6 in rheumatoid arthritis synovium is significantly related to the severity of synovitis and the number of inflammatory cell infiltration [10]. During the progression of knee osteoarthritis, further damage to cartilage leads to the aggravation of knee osteoarthritis. Compound Duzhong Jiangu Granules can effectively reduce the production of TRAF6, thereby further delaying the development of knee osteoarthritis. Proinflammatory cytokines IL-1β and TNF-α are the main causes of arthritis pain. IL-1β can stimulate the production of COX-2, iNOS, etc., further activate the inflammatory pathway and induce inflammatory pain [11]. Compound Duzhong Jiangu Granules can effectively reduce the production of pro-inflammatory factor IL-1β, so it is proved that Compound Duzhong Jiangu Granules have a good analgesic effect.

In summary, in this experiment, guinea pigs were treated with knee arthritis modeling and compound Duzhong Jiangu granules were used for gavage treatment. Through transcriptome sequencing analysis of bone marrow and cartilage tissues, the differences in gene expression of GZMK, Jchain, Igκc, IGHV3-74, IGHV3-11, IGHV4-1, CCLS, and KGKVI-39 were obtained, which provided a certain basis for the treatment of knee osteoarthritis with traditional Chinese medicine. Through WB verification, it was found that TRAF6, MIP-1β, and IL-1β in cartilage were significantly reduced. Compound Duzhong Jiangu Granules can effectively delay the progress of knee osteoarthritis by changing the expression of some genes in the treatment of knee osteoarthritis and providing some ideas for the treatment of knee osteoarthritis with traditional Chinese medicine.

References


21. Xiong A, Xiong RP, Peng Y, et al. Establishment and characteristic analysis of a model of knee fibroblast-like synoviocytes lipopolysaccharide-induced Sprague-Dawley rats. *Acta Lab Anim Sci.* 2020;28(4):436–446. Available at: https://kns.cnki.net/kcms2/article/abstract?v=HbouJJBuTKtQg2Ep7-Dk8BIC49u5AIh9gq3MHpOZt0P56TwJkSE02EQ1xAAAmywvstv854sRC5vKvQ7mUH2zcphwOvlkJJTwJF/CXbPpwhAGehYdrBvdD3w6Wtms1M_sk7EsebVFw-lu7z2NA==&uniplatform=NZKPT&language=CHS