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Effects of Radix Achyrandii Polysaccharide on Cartilage Morphology of Knee Osteoarthritis in Obese Mice Induced by High-fat Diet

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Abstract: Objective: To study the pathological changes of articular cartilage in the process of osteoarthritis induced by high-fat diet and the mechanism of Radix achyrandii polysaccharide on osteoarthritis cartilage, and to explore the protective effect of Achyrannosus achyrannosus polysaccharide on knee osteoarthritis cartilage, so as to provide effective experimental evidence for clinical treatment of knee osteoarthritis. Methods: OA model induced by anterior cruciate ligament transection was established in obese rats induced by high-fat diet. Thirty C57BL/6 J mice were randomly divided into normal diet group (CON), high fat diet group (OA) and high fat diet plus Radix achyrandii polysaccharide (RAP). The CON group was fed with a basal diet, and the OA and RAA groups were fed with a high-fat diet. After 8 weeks of feeding, the RAP group was treated with polysaccharide (200 mg/kg·d) by gavage once a day for 4 weeks, and the blank group and the model group were given the same volume of normal saline. At the end of the experiment, the serum levels of TG, TC, HDL-C and LDL-C were detected by biochemical methods. The activity of superoxide dismutase (SOD) and the levels of malondialdehyde (MDA), tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were measured by ELISA. H&E staining was used to observe the pathological changes of knee cartilage. Results: Compared with the CON group, the OA group had significantly higher serum levels of TG, TC, LDL-C, MDA, TNF-α and IL-1β, and significantly lower serum HDL-C level and SOD activity. The results of histopathological examination showed that there were pathological changes of cartilage tissue damage in OA group. Compared with the model group, the related indicators of the RA group were improved to varying degrees. Conclusion: Radix achyrandii polysaccharide can protect articular cartilage by improving OA cartilage damage caused by anterior cruciate ligament transection in obese rats, regulating blood lipid related indicators, and inhibiting inflammatory response and oxidation reaction.

Keywords: Radix achyrandii polysaccharide, High fat; Blood lipid; Osteoarthritis.

1. Introduction

Osteoarthritis (OA) is the most common arthritis disease and the main cause of disability and pain in adult patients, which can cause functional impairment and decreased quality of life[1]. It is a chronic degenerative joint disease caused by multiple factors, which mainly involves articular cartilage destruction and simultaneously involves periarticular bones, muscles, ligaments and synovium. Current studies have shown that OA joint degeneration is the result of the joint action of biochemical factors and mechanical stress[2]. The incidence is high in middle-aged and elderly people, especially those with obesity. In the United States, the incidence of knee osteoarthritis (KOA) among people aged 60 years and over is about 10% in men and 13% in women, and it continues to increase with the increase of age and the prevalence of obesity[3]. It is expected that by 2020, More than 50 million people in the United States will suffer from this disease, and it will become an important factor causing disability and activity limitation for people over the age of 40[4]. From the perspective of pathogenic characteristics, OA is most common in weight-bearing joints such as hip and knee, and is mainly characterized by joint pain, limited activity and degeneration of articular cartilage[5]. In addition to articular cartilage, the affected parts also include synovial membrane, ligament, meniscus and periarticular muscles[6]. At present, there is no unified understanding of the pathogenesis of OA, which is greatly affected by "modern environmental" factors such as obesity, metabolic syndrome, diet change, sedentary lifestyle or improper exercise[7]. The traditional view is that OA is only a joint disease caused by mechanical stress changes caused by aging and sports trauma, but a large number of epidemiological and biological data show that[8] OA is closely related to metabolic and inflammatory factors[9]. From the related studies of obesity and OA, it can be seen that both the external mechanical stress factors of obesity and the intrinsic lipid metabolism and other factors have a certain correlation with the pathological changes of OA synovial membrane and cartilage, inflammatory response and pain[10].

Chinese herbal medicine has definite curative effect, few side effects and overall regulation effect of multiple targets in the prevention and treatment of OA[11]. Studies have shown that polysaccharide from Radix achyrandii can not only enhance natural killer cell-mediated cytotoxicity and promote the proliferation of spleen cells, but also increase the phagocytic function of macrophages and the production of nitric oxide. Radix achyrandii polysaccharide can reduce inflammatory response by inhibiting the secretion of inflammatory factors, and can play an important role in the body's antioxidant[12]. However, whether Radix achyrandii polysaccharide exerts its protective effect on OA through oxidative stress is poorly studied. Therefore, in this study, knee osteoarthritis was induced by high-fat diet in C57BL/6 male mice, so as to establish an osteoarthritis model, observe the morphological changes of knee cartilage and explore the protective effect of independent Radix achyrandii polysaccharide on knee osteoarthritis cartilage, in order to provide effective experimental evidence for clinical treatment

of metabolic knee osteoarthritis.

2. Materials and Methods

2.1. Reagents

Radix achyrandii polysaccharide was purchased from Shanghai Puzhen Biotechnology Co., LTD. Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) biochemical kits were purchased from Nanjing Jiancheng BioEngineering Institute. Serum superoxide dismutase (SOD) activity, malondialdehyde (MDA) concentration and interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Shanghai Bangyi Biotechnology Co., LTD. Hematoxylin-eosin staining (HE) staining kit was purchased from Shanghai Biyuntian Biotechnology Co., LTD. RIPA tissue cell rapid lysate was purchased from Beijing Solaibao Technology Co., LTD. (item number: T8220). NC membranes were purchased from Merck millipore (HATF00010); Luminescent solution was purchased from Millipore (product number: WBKLS0100); Broad-spectrum secondary antibody was purchased from Shanghai Changdao Biotechnology Co., LTD. (item number: D-3004). MPP-3 antibody (product number: ab52915), TIMP-1 antibody (product number: ab221435), and GAPDH antibody (product number: ab8245) were purchased from Abcam.

2.2. OA model and group administration

Thirty SPF male Sprague-Dawley rats, weighing 180-220 g, were grown at room temperature of (24±2)°C with half light and half dark. The rats were free to eat and drink. Adaptive feeding was performed for 1 week, and during the adaptive feeding phase, all rats were given a common rodent diet. At the end of the adaptation feeding, the rats were given high-fat diet (fat 45%, carbohydrate 36%, protein 19%). At the fourth week, when the body weight was more than 29% of the rats given normal diet, 40 rats were selected to meet the standard. At the same time, the blank group was fed with normal diet (n=10). The OA model was established by anterior cruciate ligament transection and medial meniscectomy. The rats were anesthetized and the hair of the right knee was clipped. The anterior cruciate ligament was cut. After surgery, the joints were washed with normal saline and the skin was sutured. In the blank group, the anterior cruciate ligament was exposed but not cut. After the operation, the obese OA model rats were randomly divided into model group (OA) and Radix achyrandii polysaccharide group (200 mg/kg, RAP), with 10 rats in each group. The rats in the OA model group and the blank group were given the same volume of normal saline, and the rats in the RAP group were given the corresponding drugs by gavage once a day for 4 weeks. This continued until the end of the 8-week experiment. All animals were fasted for 12 h before the last administration, and mice were anesthetized 1.5 h after the last administration and blood samples were collected. The target knee tissues were collected after the rats were euthanized with carbon dioxide.

2.3. Serum biochemical indicators

Serum TG, TC, HDL-C and LDL-C levels were measured by biochemical analyzer according to the instructions of each kit. The activity of SOD and the levels of MDA, TNF-α and IL-1β in serum were detected by ELISA kit.

2.4. Histopathological examination

The morphological changes of articular cartilage were observed by H&E staining，the experimental procedure was decalcification, paraffin embedding,slice-dye-observation-immobilization, and the operation was strictly performed according to the operating instructions. The cartilage tissue of 5 rats in each group was randomly taken, and the protein expressions of MMP-3 and TIMP-1 were detected by Western blotting.

2.5. Data statistics and analysis

Data were analyzed and plotted by R software. The experimental data were expressed as mean ± standard deviation, and ordinary one-way analysis of variance was used between groups. $P<0.05$ indicates a significant difference, $P<0.01$ indicates a very significant difference, $P<0.001$ was considered to be highly significant.

3. Results

3.1. Effects of RAP on blood lipids in OA rats

As shown in Fig.1, compared with the blank group, the serum levels of TG, TC and LDL-C in the model group were significantly increased, and the level of HDL-C was significantly decreased ($P < 0.05$). Compared with the model group, the serum levels of TG, TC and LDL-C were significantly decreased, and the level of HDL-C was significantly increased in the RAP group ($P < 0.05$).

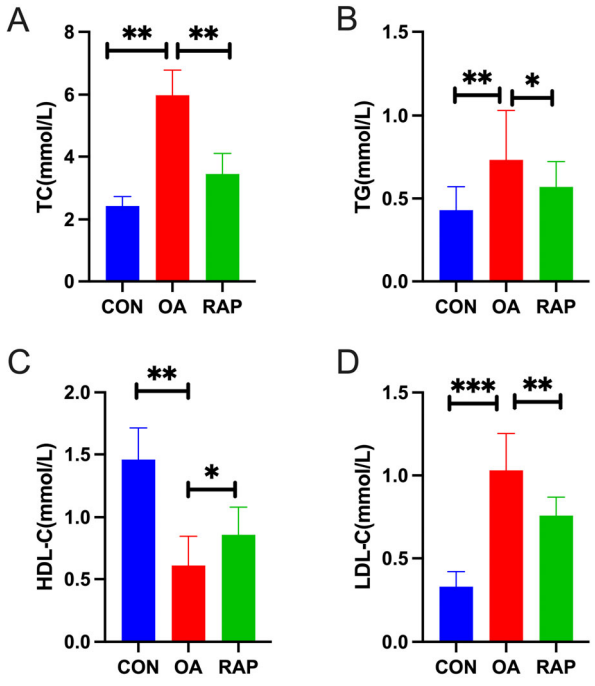

Figure 1. Effects of RAP on blood lipids in rats

3.2. Effects of RAP on antioxidant and anti-inflammatory properties in OA rats

Compared with the blank group, the serum SOD activity of the model group was significantly decreased, and the levels of MDA, TNF-α and IL-1β were significantly increased. Compared with the model group, the activity of SOD was significantly increased, and the levels of MDA, TNF-α and IL-1β were significantly decreased in the RAP group (Fig. 2).

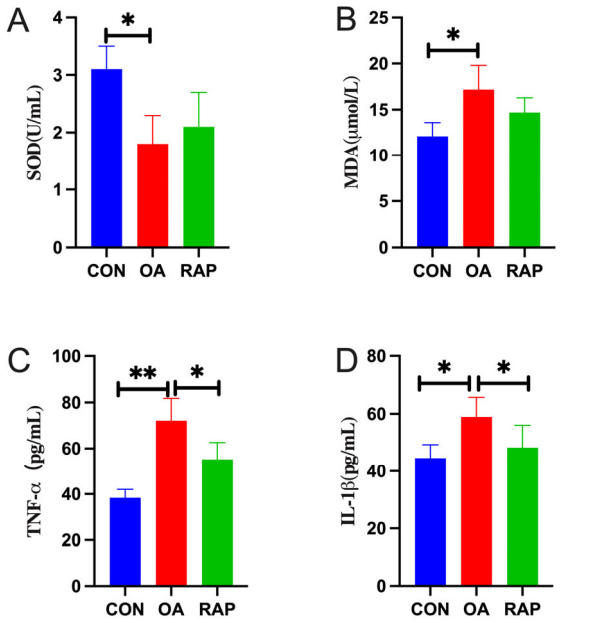

Figure 2. Effect of Artemisia selengensis cellulose on blood lipids in diabetic mice

3.3. Effect of RAP on histopathology of articular cartilage in OA rats

In the blank group, the cartilage tissue cells were closely arranged, the cartilage surface was smooth and the tide line was clear. In the model group, the staining around the chondrocytes became light, the cells were arranged loosely, and the tide line was blurred or broken. Compared with the model group, the arrangement of chondrocytes and the clarity of the tideline in the RAP group were improved, although the cell accumulation and the surface were blurred, but the continuity was acceptable.

3.4. Effects of RAP on the expression of MMP-3 and TIMP-1 protein in OA rats

Compared with the blank group, the expression of MMP-3 and TIMP-1 protein in articular cartilage tissue of the model group was significantly increased. Compared with the model group, the protein expression of MMP-3 and TIMP-1 in the RAP group was significantly decreased.

4. Summary

Overweight and obesity are one of the important risk factors for the occurrence and development of knee OA. Obesity can lead to increased mechanical stress of the joint, abnormal degradation of articular cartilage, bone loss, and chronic inflammatory response. Obese knee OA is closely related to inflammation and oxidative stress, which is an important factor involved in cartilage degradation leading to cartilage degeneration. Inflammatory cytokines such as IL-1β and TNF-α mainly stimulate the production of special matrix metalloproteinases (MMPs) to cause the degradation of matrix, and can induce the formation of inflammatory mediators by stimulating the proliferation and degeneration of synovial cells. It can promote the secretion of metalloproteinases in synoviocytes, inhibit or interfere with the expression of chondrocytes and phenotypes. When the duration of obesity is longer, the antioxidant sources are reduced, resulting in decreased SOD activity as well as increased MDA levels. The results of the present study showed that RAP increased SOD activity and decreased MDA levels. It is suggested that RAP can regulate serum inflammatory changes and oxidative stress injury in model rats, thereby improving the symptoms of obese OA.

TIMP-1 and MMP-3 are important cytokines in the pathogenesis of OA. In the pathogenesis of OA, MMPs play a decisive role in the imbalance of synthesis and degradation of articular cartilage extracellular matrix, and MMP-3 gene is highly expressed in patients with knee OA. In this study, the protein expression of cartilage tissue was detected, and it was found that compared with the model group, the RAP treated group had a significant inhibitory effect on the overexpression of MMP-3 and TIMP-1 protein in the model mice. These results indicate that RAP can regulate the activity of MMP-3 and the expression of TIMP-1 synchronously, maintain their intrinsic balance, and promote the synthesis of knee articular cartilage in obese rats.

In conclusion, Achyrantha bidentata polysaccharide has lipid-lowering, anti-inflammatory and anti-oxidative effects on knee OA in obese rats, and it can improve OA induced by obesity and ligament injury by regulating the balance of MMP-3 and TIMP-1.

5. Conflicts of Interest

The authors declare that they have no conflict of interest.

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