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·临床研究·

金天格胶囊对膝骨关节炎患者关节液中 MMP-3、TIMP-1、IL-1β、TGF-β1 水平的影响

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中图分类号:R966;R684.3 **文献标识码**:A **文章编号**:1006-7108(2015)04-0442-05

摘要:**目的** 探讨金天格胶囊对膝骨关节炎(knee osteoarthritis,KOA)患者关节液中基质金属蛋白酶-3(MMP-3)、基质金属蛋白酶组织抑制剂-1(TIMP-1)、白细胞介素-1β(IL-1β)及转化生长因子-β1(TGF-β1)的影响。**方法** 选取 90 例 KOA 患者,分别在治疗前、治疗 1 个疗程、治疗 2 个疗程后,对患者进行 WOMAC 量表评分,评估患者膝关节功能。分别抽取患者膝关节腔关节液,进行酶联免疫吸附法(ELISA),测定关节液中 MMP-3、TIMP-1、IL-1β、TGF-β1 的含量。**结果** (1)服用金天格胶囊后,患者 WOMAC 评分降低,膝关节功能改善。(2)与治疗前相比,治疗后关节液中 MMP-3、IL-1β 含量都有所降低,TIMP-1、TGF-β1 含量都有所增加,差异具有统计学意义($P<0.01$)。(3)随着服用金天格胶囊疗程的增加,膝关节液中 MMP-3、IL-1β 含量逐渐下降,TIMP-1、TGF-β1 的含量逐渐升高,组间差异具有统计学意义($P<0.01$)。**结论** 金天格胶囊可以降低 KOA 患者关节液中 MMP-3、IL-1β 水平,升高 TIMP-1、TGF-β1 的水平,从而起到保护关节软骨、改善 KOA 患者膝关节功能的作用。

关键词:金天格胶囊;膝骨关节炎;基质金属蛋白酶-3(MMP-3);基质金属蛋白酶组织抑制剂-1(TIMP-1);白细胞介素-1β(IL-1β);转化生长因子-β1(TGF-β1)

Effects of Jintiange capsule on MMP-3, TIMP-1, IL-1β, and TGF-β1 levels in synovial fluid of patients with knee osteoarthritis

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Abstract: **Objective** To evaluate the effect of Jintiange capsule on the levels of MMP-3, TIMP-1, IL-1β, and TGF-β1 in synovial fluid of patients with knee osteoarthritis (KOA). **Methods** Ninety KOA patients were involved. WOMAC scores were used to evaluate the knee joint function before the treatment and after one and two courses of the treatment. The synovial fluid in knee joint was collected and the levels of MMP-3, TIMP-1, IL-1β, And TGF-β1 in the synovial fluid were detected using ELISA method. **Results** 1) After oral intake of Jintiange capsule, WOMAC scores of the patients decreased significantly, and the knee joint function improved. 2) The levels of MMP-3 and IL-1β in synovial fluid after the treatment gradually decreased comparing to those before the treatment, and the levels of TIMP-1 and TGF-β1 in the synovial fluid gradually increased ($P<0.01$). 3) Following the increase of the time course of Jintiange treatment, the levels of MMP-3 and IL-1β in synovial fluid decreased and the levels of TIMP-1 and TGF-β1 increased. The differences between the groups were significant ($P<0.01$). **Conclusion** Jintiange capsule can markedly decrease the levels of MMP-3 and IL-1β, and increase the levels of TIMP-1 and TGF-β1 in synovial fluid. Thus, Jintiange Capsule can protect the articular cartilage and improve the knee joint function of KOA patients.

Key words: Jintiange capsules; Osteoarthritis; MMP-3; TIMP-1; IL-1β; TGF-β1

Knee osteoarthritis (KOA) is one of the common degenerative diseases of joint surgery, pathologically characterized by progressive retrograde degeneration of articular cartilage, proliferation of joint edge and reactive change of subchondral bone substance. The pathological changes implicate the whole joint

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progressively over time, so that the knee joint function lost eventually. The clinical manifestations of KOA commonly are arthralgia and activity obstacle, seriously affecting the daily life of patients. With the progress of the population aging in our country, the incidence of KOA raises year by year.

Recent researches suggest that the degradation of cytokines on cartilage extracellular matrix is the main pathogenesis of KOA. The cartilage includes cartilage cells and extracellular matrix (ECM), and the metabolic process of cartilage is very complicated. The ECM mainly consists of collagen type II and proteoglycan, maintaining dynamic equilibrium of degradation and synthesis. MMP-3, a kind of matrix metalloproteinases (MMPs), is one of the main enzymes of KOA cartilage degradation, and can degrade many kinds of compositions of cartilage ECM[1-2]. TIMP-1, the specific inhibiting factor of MMP-3, can inhibit the activity of MMP-3. In addition, IL-1β, a kind of pro-inflammatory cytokines, not only can destroy the cartilage cells, but also degrade cartilage ECM by elevating the MMP-3 level[3]. But TGF-β1 is a recognized suppression factor of inflammation, and can significantly reduce the IL-1β. The early stage research shows that Jintiange capsules have certain protective effects on cartilage, but the mechanism is unknown. Thus, our experiment is aim to observe the effect of Jintiange capsules on levels of MMP-3、TIMP-1、IL-1β and TGF-β1 in synovial fluid of KOA patients and take the correlation analysis, to explore the protection mechanism of Jintiange capsules on cartilage.

1 CLINICAL MATERIALS

1.1 General Data

A total 90 patients were diagnosed as KOA in Department of Orthopedics of East Qingdao municipal hospital from February 2013 to February 2014. There were 27 males and 63 females aged 55 ~ 70, 59.4 on average, with illness course 7 ~ 120 months, 6.8 years on average.

1.2 Standards for Diagnosis and Inclusion

1.2.1 Standard for diagnosis: The patients were diagnosed according to "American College of Rheumatology" (ACR) standards of knee osteoarthritis in 1989[4]: (1) joint pain lasts over 14 days in a month before treatment; (2) sound of bony friction can be heard; (3) osteophyte can be found around bone edge of knee joint by X ray; (4) swelling around knee joint; (5) morning stiffness lasts less than 30 minutes; (6) patients are over 40 years old. Whoever conforms to 1、3 or 1、2、4、5、6 can be diagnosed.

1.2.2 Standard for inclusion: Patients who had the following items were included: (1) conformed to standards for diagnosis; (2) age ranges from 55 to 70 years; (3) not taken non-steroid anti-inflammatory drug、glucocorticoids、chondroprotective agents or such drugs; (4) will to obey our treatment plan or not accept other treatment plans.

1.3 Standard for Exclusion

Patients had serious cardiovascular disease, cerebrovascular disease and primary diseases of the liver, kidney or digestive system or mental disease. Patients had secondary arthritis, such as autoimmune arthritis, trauma, etc. Patients had bone tuberculosis, bone tumors or other bone metastatic cancer. Women were in pregnancy or lactation. Patients had taken glucocorticoids drugs recently. Patients did't obey the treatment plan.

2 METHODS

2.1 Therapeutic Method

Patients were orally given Jintiange capsules (Ginwa enterprise Inc. China, box of 24 capsules 0.4 g), 3 capsules 3times daily. Two months were taken as a course of treatment.

2.2 WOMAC Index Assessment

According to the WOMAC index[5], the WOMAC scores were compared to evaluate the knees' function before the treatment, after one course of treatment and two courses of treatment. The assessment included three aspects: pain, stiffness, physical function, totally 24 titles. The score of every title is 0 ~ 4 points, and the higher the score, the severer the symptom was.

2.3 Synovial Fluid Collection and ELISA

The patient was in supine position, and legs unbent. The superior lateral quadrant of patella was chosen as puncture point. After regular disinfection

and local infiltration anesthesia, punctured into the joint cavity with size seven needle and drew 1 ~ 2ml synovial fluid. This operation should be taken three times for every patient, before the treatment, after one course of treatment and two courses of treatment. Then, the synovial fluid was injected into centrifuge tubes and centrifuged at 4000 r/min, for 10 min. The supernatant was kept at -20℃. Finally, MMP-3、TIMP-1、IL-1β and TGF-β1 levels were detected with ELISA method.

2.4 Statistical Analysis

The data were analyzed using SPPS 19.0 software and expressed as mean ± SD. Single factor variance analysis of repeated measurement was performed. *P* values of less than 0.01 were considered statistically significant.

3 RESULTS

3.1 Comparison of changes in WOMAC index

As shown in Table 1, there was significant difference ($P < 0.01$) in the WOMAC scores of patients after one course of treatment compared with before treatment. With the increase of treatment course, the WOMAC scores dropped more obviously.

表 1 治疗前后 KOA 患者 WOMAC 评分比较($\bar{x} \pm s$)

Table 1 Comparison of WOMAC scores before and after the treatment ($\bar{x} \pm s$)

时间 Time	WOMAC 评分 WOMAC Score
治疗前 Before treatment	60.07 ± 10.01
治疗 1 个疗程后 After one course of treatment	45.16 ± 9.99 *
治疗 2 个疗程后 After two courses of treatment	36.18 ± 9.90 *▲

注：* 与治疗前比较，$P < 0.01$；▲ 与治疗 1 个疗程后比较，$P < 0.01$。

Note: * $P < 0.01$ as compared with the datum before treatment; ▲ $P < 0.01$ as compared with the datum after one course of treatment.

3.2 Comparison of Levels of MMP-3、TIMP-1、IL-1β and TGF-β1 in Synovial Fluid

As shown in table 2, compared with before treatment, the levels of MMP-3、IL-1β was gradually decreased, and the levels of TIMP-1、TGF-β1 was gradually increased after one and two course of treatment. The differences was significant ($P < 0.01$).

表 2 不同阶段关节液中 MMP-3、TIMP-1、IL-1β、TGF-β1 含量变化($\bar{x} \pm s$)

Table 2 Comparison of levels of MMP-3, TIMP-1, IL-1β, and TGF-β1 in the synovial fluid before and after the treatment ($\bar{x} \pm s$)

时间 Time	MMP-3 (ng/ml)	TIMP-1 (ng/ml)	IL-1β (ng/ml)	TGF-β1 (pg/ml)
治疗前 Before treatment	187.81 ± 11.16	296.62 ± 18.61	127.87 ± 7.06	686.14 ± 61.46
治疗 1 个疗程后 After one course of treatment	117.58 ± 16.87 *	383.78 ± 12.61 *	97.97 ± 9.02 *	787.99 ± 69.15 *
治疗 2 个疗程后 After two courses of treatment	82.82 ± 15.66 *▲	420.47 ± 15.30 *▲	75.84 ± 8.79 *▲	887.15 ± 63.04 *▲

注：* 与治疗前比较，$P < 0.01$；▲ 与治疗 1 个疗程后比较，$P < 0.01$。

Note: * $P < 0.01$ as compared with the datum before treatment; ▲ $P < 0.01$ as compared with the datum after one course of treatment.

4 DISCUSSION

In recent years, it has made great progress in the aspects of KOA's pathogenesis and pathological changes. It is generally accepted that degradation enzymes of cartilage and cytokines are two important factors involved in pathogenesis of KOA. Among them, MMPs and IL-1β are the most important enzyme and cytokine, having the most closely relationship with the growth, differentiation of cartilage cells and metabolism of cartilage.

The cartilage consists of cartilage cells and ECM. The balance of ECM is an important condition for activity and function of cartilage, which mainly consists of collagen type II and proteoglycan. Researches show that MMPs, which play an important role in the

destruction of cartilage, are the major enzymes during the process of cartilage destruction[6]. It is a family of proteolysis enzymes relied on zinc ions, including collagenases, gelatinases, tromelysins and so on. The expression of tromelysin-1, namely MMP-3[7], increases in pathological cartilage, synovium and subchondral bone. It can degrade proteoglycan, collagen type III, IV, IX, XI and other protein substrates in ECM[8]. Moreover, MMP-3 also participates in the activation of interstitial collagenase, which can degrade collagen type II. Thus, MMP-3 influence the degradation of collagen type II indirectly. Thereby, MMP-3 is now regarded as the most important protease which inducts the cartilage degradation and the indicator of severity and prognosis of KOA[9]. In the other aspect, studies have identified that TIMPs, a kind of inhibitor of MMPs, can suppress the activity of MMPs. TIMP-1, specific physiological inhibitor of MMP-3, exists generally in tissues and humor, which can be produced by synoviocyte, chondrocyte, macrophage and connective tissue. It can be induced by cytokines to prevent the degradation of cartilage, and promote the cells growth through binding with the pattern recognition receptors[10]. Normally, it maintains dynamic balance between MMPs and TIMPs. Once the balance is broken, plenty of ECM will be degrade, which leads to the destruction of environment and collagen network around cartilage cells, and KOA occurs.

It has be confirmed that the level of IL-1β in synovial fluid of KOA patients increases significantly, and it is positively correlated with the severity of KOA[11]. IL-1β, a kind of cytokine produced by chondrocyte, synoviocyte and macrophage, is the initiating factor of inflammation. Studies have reported that the quantity of IL-1β receptors on the surface of the cartilage cells of KOA patients is twice than normal cartilage cells[12], and this makes the cartilage cells more sensitive to IL-1β. IL-1β can lead to degradation of proteoglycan and destruction of cartilage cells, inhibit proteoglycan and collagen type II which have cartilage properties, and contrarily promote collagen type I generation which has fibroblast properties. Gradually, the cartilage cells degenerate which leads to cartilage defect and progression of KOA. Besides, IL-1β promotes synovial cells to excrete PGE_2、NO and collagenase to generate strong proinflammatory effect, and induces the absorption of synovial inflammation and bone. Furthermore, IL-1β can promote the production of matrix metalloproteinases preferment and inhibit the production of matrix metallo-proteinase inhibitor[13]. A recent study has found that human articular cartilage cells stimulated by IL-1β can generate and release more MMP-1, MMP-3 and MMP-13[3]. TGF-β1 is a class of protein polypeptide which can regulate cell differentiation, proliferation and promote synthesis of cell matrix. TGF-β1 plays an important role in the process of cartilage formation and repair. It has been verified that TGF-β1 can promote the proliferation and differentiation of cartilage cells, and induce the synthesis of them[14]. Under the effect of TGF-β1, cartilage cells increase the synthesis of proteoglycan, suppress the degradation of proteoglycan. Thus, it maintains relatively stable concentration of protein polysaccharide in cartilage ECM. Other studies also found that TGF-β1 can reduced the destructive effect of IL-1β on cartilage cells though lowering synthesis of IL-1β receptors and increasing expression of interleukin receptor antagonist proteins. Besides, TGF-β1 can increase the level of proteoglycan and TIMPs, inhibit the synthesis of MMP-3 to protect cartilage[15].

Jintiange capsules compose of artificial tiger bone powder processed by the animal bones. The capsules contain various essential trace elements and amino acids, also multiple organic ingredients, such as collagen, scorpion analgesic peptide, bone morphogenetic protein, bone growth factors, polysaccharide and so on. These ingredients can stimulate the proliferation of cartilage cells and strengthen their activation directly, so that the capsules can promote to repair the surface of articular cartilage and improve the function of knee.

Our experiment showed that the WOMAC scores of KOA patients treated with Jintiange capsules declined compared with those in the model group, which represented the improvement of knees' function of these patients. At the same time, the level of MMP-3 and IL-1β in synovial fluid decreased, and the level of TIMP-1、TGF-β1 increased. These results proved that MMP-3 and IL-1β had destructive effect on cartilage in

the progression of KOA, while TIMP-1 and TGF-β1 had protective and restorative effect oppositely. With the increase of treatment course of Jintiange capsules, the function of knees further improved, the level of MMP-3 and IL-1β in synovial fluid decreased, and the level of TIMP-1、TGF-β1 increased more obviously. These results indicated that Jintiange had following effects: lower the level of cartilage degradation enzymes and cytokines directly or indirectly; relieve the synovial inflammation; reduce the secretion of various cell active factors; relieve the damage of cartilage matrix-degrading enzymes and cytokines on cartilage matrix; improve the metabolism of cartilage matrix. In conclude, Jintiange capsules can protect the cartilage, and improve the functions of knee.

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