Cartilage oligomeric matrix protein and matrix metalloproteinase-3 expression in the serum and joint fluid of a reversible osteoarthritis rabbit model

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ABSTRACT. The main pathological characteristic of osteoarthritis (OA) is cartilage damage. We explored cartilage oligomeric matrix protein (COMP) and matrix metalloproteinase-3 (MMP-3) changes during articular cartilage injury and repair. Rabbits were randomly divided into the following: a blank control group; groups M1, M2, and M3, in which breaking was performed for 2, 4, and 6 weeks, respectively; and groups L1, L2, and L3, in which breaking was discontinued for 2, 4, and 6 weeks, respectively, following a 4-week recovery period. There are 7 rabbits in each group. The degree of cartilage damage in each group was scored (OA score). An enzyme-linked immunosorbent assay was used to detect COMP and MMP-3 levels in serum and joint fluid. The OA scores were 3.89 ± 2.31, 7.21 ± 2.31, and 10.88 ± 2.08 points in groups M1, M2, and M3, respectively ($P < 0.05$). COMP and MMP-3 levels were significantly

higher in groups M1, M2, and M3 than in C. The OA score improved significantly following the 4-week recovery period ($P < 0.05$). COMP and MMP-3 levels began to decrease as the time following discontinuation of breaking increased, but were higher than in the control ($P < 0.05$). MMP-3 and COMP levels were correlated with OA score ($r > 0.7$, $P < 0.05$). COMP and MMP-3 levels were correlated between joint fluid and serum ($r = 0.899$, $r = 0.874$, $P < 0.05$, respectively). Long-term joint breaking can cause articular cartilage damage. Doing some activites after the process can promote self-repair of articular cartilage. COMP and MMP-3 levels were associated with articular cartilage destruction and repair.

Key words: Osteoarthritis; Cartilage oligomeric matrix protein; Reversible; Matrix metalloproteinase-3

INTRODUCTION

Osteoarthritis (OA) is a degenerative disease that is associated with obesity, age, trauma, strain, joint deformity, and congenital anomalies. Its clinical manifestation includes slow development of joint pain, stiffness, tenderness, joint swelling, joint deformity, and restricted movement (Lahm et al., 2004; Fernandes et al., 2007) OA is a common joint disease with the main pathological process being articular cartilage matrix degradation. The critical factor of OA disease development is the change in cartilage cell metabolism. Articular cartilage destruction mainly happens in two ways: the cartilage extracellular matrix is degraded by the cartilage cells themselves or the cartilage extracellular matrix is damaged by invasive inflammatory cells, pannus tissue, or inflammatory synovium through the joint synovial fluid. The main factor in cartilage damage is the enzymatic degradation of the extracellular matrix (Arai et al., 2008; Posey and Hecht, 2008). It has been suggested (Posey and Hecht, 2008) that matrix metalloproteinases (MMPs) play an important role in the injury of articular cartilage, while MMP-3 is one of the most important enzymes among the articular cartilage matrix-degrading enzymes. Cartilage oligomeric matrix protein (COMP) is a type of biomarker that can reflect OA progression. It has been found that COMP is associated with the severity of OA and its level increases significantly in OA patients' serum, joint fluid, and urine, which may be released from the joint fluid (Hunter et al., 2007; Valdes et al., 2014). Current clinical diagnosis and evaluation of OA mainly depend on factors like clinical manifestations, imaging, and arthroscopy. However, all of these checks lag behind the pathological changes, and are based on cartilage structural damage. OA is difficult to diagnose before articular cartilage damage. Repair response can be observed in early OA, and an *in vitro* study (Sharif et al., 2004) has shown that early pathological changes can be reversed. If an early diagnosis based on the biochemical metabolism can be made before the morphological changes take place, better treatment could be applied to slow down or reverse the disease. Therefore, monitoring specific biological markers in the body fluids can indirectly reflect the condition of OA development and the extent of cartilage lesions. COMP and MMP-3 level changes are of great significance in the early diagnosis and drug treatment of OA. This study aims to investigate the correlation between COMP and MMP-3 expression and the degree of cartilage injury using a reversible osteoarthritis rabbit model produced by limb breaking.

MATERIAL AND METHODS

Materials

Experimental animals and grouping

New Zealand rabbits (male, 6 months old, weighting 2.0-2.5 kg) were provided by the Hubei University of Chinese Medicine animal experiment center (license SYXK-2013-0025). The rabbits were specific-pathogen-free laboratory animals with no knee joint trauma or swelling, and normal buckling function. They were randomly divided into the following: a blank control group (group C); groups M1, M2, and M3, in which breaking was performed for 2, 4, and 6 weeks, respectively; and groups L1, L2, and L3, in which breaking was discontinued for 2, 4, and 6 weeks, respectively, following a 4-week recovery period. There were seven rabbits in each group.

Rabbits were used for all experiments, and all procedures were approved by the Animal Ethics Committee of our hospital.

Experimental methods

Modeling

The modeling started after adaptive breeding for 1 week. Polyester polymer plaster was applied to each rabbit's right leg. The leg was broken using an unbent gypsum tube for different periods to create the osteoarthritis model (Hayami et al., 2006). The right hind limb was fixed in a hyperextension position using polyester polymer plaster. The fixed scope was 2.0-3.0 cm under the ankle to 1.0-2.0 cm under the groin. The ankle was dorsiflexed through 30-40°. The status of the rabbits' daily life and plaster fixation were monitored, and repairs were made when necessary. Two rabbits after breaking for 6 weeks were randomly selected for right leg-joint X-ray examination before modeling, and 2, 4, and 6 weeks after modeling. After killing the rabbits, the femoral knee joint and part of the cartilage tissue from the trochlea were collected for hematoxylin and eosin (HE) staining and histological examination.

Serum COMP and MMP-3 level detection

The venous blood and joint fluid were collected and centrifuged at 14000 g (r = 10 cm, 3500 r/min) for 15 min. An enzyme-linked immunosorbent assay (ELISA) was used to detect COMP and MMP-3 levels according to the manufacturer specification (Yu Ping Biotechnology Company, Shanghai).

Statistical analysis

All statistical analyses were performed using SPSS18.0 software (Chicago, IL). Measured data with normal distribution were presented as mean ± standard deviation (means ± S). One-way analysis of variance was used for multiple group comparison. The least significant difference (LSD) test was applied for comparison between groups. The Spearman method was performed for correlation analysis. $P < 0.05$ was considered to indicate a statistically significant result.

RESULTS

OA score comparison at different time points in the rabbit model

As shown in Table 1, compared with the control group, OA scores were significantly elevated after breaking for 2, 4, and 6 weeks ($P < 0.05$). The OA score increased significantly following the breaking time extension, and the OA score improved markedly following the recovery period after discontinuing breaking (4 weeks) ($P < 0.05$), but all OA scores were higher than in the blank control group. The degree of articular cartilage damage was alleviated after breaking was discontinued.

Table 1. Comparison of scores at different time points in the rabbit model (means ± S).

Group	N	Osteoarthritis (OA) score
Control	7	0.00 ± 0.00
M1	7	3.89 ± 2.31*
M2	7	7.21 ± 2.11*
M3	7	10.88 ± 2.08*
L1	7	6.86 ± 1.76*#
L2	7	6.05 ± 2.12*#
L3	7	3.35 ± 1.42*#

*Compared with control, $P < 0.05$. #Compared with M2, $P < 0.05$. Significant differences between pairs of groups, $P < 0.05$.

Joint cartilage lesion morphology observation at different time points in the rabbit model

The control group presented a smooth articular cartilage surface, aligned cartilage cells, a clear hierarchical structure, a uniform dyeing matrix, and a complete tide line. Following breaking time extension, the pathological damage to joints was aggravated. After discontinuing breaking, the joints repaired themselves and there was pathological joint damage relief. Joint damage does not develop irreversibly, and articular cartilage self-repair can restore joint cell function (Figure 1).

A smooth articular cartilage surface, alignment of cartilage cells, a clear hierarchical structure, a uniform dyeing matrix, and a complete tide line can be seen in Figure 1A. A rough articular cartilage surface, more numerous and disordered cartilage cells, a non-uniform dyeing matrix, and an incomplete tide line can be seen in Figure 1B. A rough articular cartilage surface, more cracks, more numerous and disordered cartilage cells, a non-uniform dyeing matrix, and an incomplete tide line can be seen in Figure 1C. More cracks, a rough articular cartilage surface, necrotic and disordered cartilage cells surrounding the cracks, a non-uniform dyeing matrix, and an incomplete tide line can be seen in Figure 1D. Improved cartilage cell surface smoothness, cell arrangement uniformity, and fracture reduction can be seen in Figures 1E, F, G.

Joint fluid and serum COMP and MMP-3 expression at different time points in the rabbit model

Joint fluid and serum COMP and MMP-3 levels were significantly higher in groups M1, M2, and M3 than in the control group. They increased significantly following the braking time

extension (P < 0.05). Joint fluid and serum COMP and MMP-3 levels began to decrease following the recovery time after discontinuing breaking, but were higher than in the blank control (P < 0.05). COMP and MMP-3 levels correlated in the joint fluid and serum at different time points (r = 0.899, r = 0.874, P < 0.05, respectively) (Tables 2 and 3).

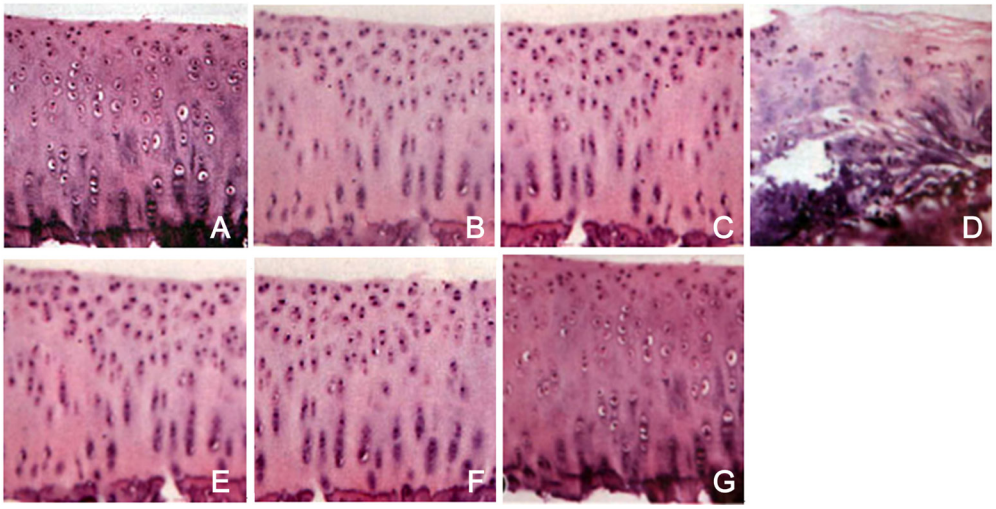

Figure 1. Joint cartilage lesion morphology observation at different time points in the rabbit model. **A.** Control group, hematoxylin and eosin stain (HE) 200X. **B.** M1 group, HE 200X. **C.** M2 group, HE 200X. **D.** M3 group, HE 200X. **E.** L1 group, HE 200X. **F.** L2 group, HE 200X. **F.** L3 group, HE 200X.

Table 2. Serum cartilage oligomeric matrix protein (COMP) and matrix metalloproteinase-3 (MMP-3) expression at different time points in the rabbit model (means ± S, N = 7).

Group	COMP (μg/L)	MMP-3 (μg/L)
Control	3.34 ± 0.31	1.58 ± 0.36
M1	3.68 ± 0.15*	1.97 ± 0.54*
M2	3.82 ± 0.21*	2.68 ± 0.44*
M3	3.98 ± 0.38*	3.45 ± 0.37*
L1	3.72 ± 0.22*#	2.54 ± 0.31*#
L2	3.65 ± 0.17*#	2.25 ± 0.33*#
L3	3.48 ± 0.26*#	1.82 ± 0.42*#

*Compared with control, P < 0.05. #Compared with M2, P < 0.05.

Table 3. Joint fluid cartilage oligomeric matrix protein (COMP) and matrix metalloproteinase-3 (MMP-3) expression at different time points in the rabbit model (means ± S, N = 7).

Group	COMP (μg/L)	MMP-3 (μg/L)
Control	5.87 ± 1.21	4.58 ± 1.06
M1	12.74 ± 2.35*	28.67 ± 3.27*
M2	20.88 ± 3.11*	38.94 ± 3.14*
M3	30.45 ± 2.78*	49.45 ± 3.35*
L1	15.76 ± 2.45*#	30.54 ± 3.21*#
L2	11.43 ± 2.12*#	21.28 ± 2.63*#
L3	8.66 ± 2.06*#	15.82 ± 2.22*#

*Compared with control, P < 0.05. #Compared with M2, P < 0.05.

Correlation analysis of OA score with COMP and MMP-3 at different time points

The OA score presented linear correlation with MMP-3 and COMP expression at 2 weeks after breaking ($r = 0.716$, $r = 0.821$, $P < 0.05$, respectively), 4 weeks after breaking ($r = 0.724$, $r = 0.775$, $P < 0.05$, respectively), and 6 weeks after breaking ($r = 0.709$, $r = 0.815$, $P < 0.05$, respectively).

DISCUSSION

Iatrogenic breaks are commonly used in orthopedic treatment. Long-term breaks affect the surrounding tissue and joint function and damage the joint's mechanical and biological balance (Park et al., 2007), which triggers a chain reaction and causes articular cartilage damage. Joints may recover from joint stress and promote articular cartilage self-repair if the breaking is discontinued within a certain time. The unbent gypsum tube breaking model is reliable, has a high success rate, involves a simple operation, and indirectly acts on the joint. Compared with the operation and drug injection method, it avoids man-made damage and is not subject to surgical traumatic synovitis. It shows similar clinical pathology to articular cartilage degenerative diseases, and is suitable for OA biochemical metabolism and pathological mechanism investigation (Cui et al., 2007; Chaganti et al., 2008).

Compared with the blank control group, the OA score was significantly elevated after breaking for 2, 4, and 6 weeks ($P < 0.05$). As the breaking time increased, the OA score increased significantly. The OA scores improved markedly following the recovery time after discontinuation of breaking (4 weeks) ($P < 0.05$), but they were all higher than in the blank control group. The degree of articular cartilage damage decreased after breaking was discontinued. Articular cartilage lesions caused by long-term breaking can be seen in the articular cartilage pathological morphology. Longer breaking times correspond to more severe cartilage damage. After breaking is discontinued, articular cartilage can repair itself, showing improved cartilage cell surface smoothness, uniformity of arrangement, and fracture reduction. Recovery time extension results in more obvious cartilage improvement.

OA is a common clinical disease, the main pathological process being articular cartilage matrix degradation. The critical factor in bone arthritis development is change in the cartilage cell metabolism. Articular cartilage metabolism can be monitored by biomarkers that are released into the joint synovial fluid and further released into the urine and blood. Therefore, monitoring biomarkers is important for early prevention and treatment of OA before significant articular cartilage damage or imaging changes occur (Halász et al., 2007; Koskinen et al., 2011). Research has revealed that a variety of matrix metalloproteinases and cytokines are involved in articular cartilage destruction, which arises from a complex network of factors rather than from a single cause (Li et al., 2011; Erhart et al., 2012). MMP-3 is a protease that can degrade the cartilage matrix. Under normal circumstances, MMP-3 secretion is important for maintaining cartilage integrity. COMP is an extracellular matrix protein that is a biomarker for articular cartilage injury. The levels of COMP and MMP-3 increase in the serum of OA patients (Bobacz et al., 2003). COMP was originally found in articular cartilage, and later found in tendons, synovial membranes, and bones. It only exhibits high expression levels in the articular cartilage, and has significant tissue specificity. COMP can stabilize the cartilage collagen network by combining with collagen type II. It is released into the joint synovial fluid and blood during cartilage degradation. It is overexpressed in patients with

familial OA caused by *COL2A1* gene (type II procollagen gene) mutations. COMP changes are consistent with OA progression (Weng et al., 2010). COMP levels are correlated with the degree of pathological cartilage damage in the shark alkane chronic erosive OA rat model established using pristane (Kang et al., 2010). Our study suggested that joint fluid and serum COMP and MMP-3 levels were significantly higher in groups M1, M2, and M3 than in the blank control group. They increased significantly following the extension of the breaking time ($P < 0.05$). Joint fluid and serum COMP and MMP-3 levels began to decrease following the recovery time, but were higher than in the control ($P < 0.05$). MMP-3 and COMP levels were correlated with the OA score ($r > 0.7$, $P < 0.05$). COMP and MMP-3 levels were correlated between joint fluid and serum at different time points ($r = 0.899$, $r = 0.874$, $P < 0.05$, respectively). This indicates that serum and joint fluid COMP and MMP-3 levels are correlated with articular cartilage pathological degree in OA.

MMP-3 is generated by connective tissue cells, synovial cells, and fibroblasts. It can degrade substances like laminin, fibronectin, elastin, collagen types II, IV, and XI, basement membrane components, and extracellular proteoglycan. In particular, it is highly effective at degrading proteoglycan, thereby destroying articular cartilage stability. It can regulate the enzymatic activity of other MMPs such as MMP-1 and MMP-9, and promote collagen degradation, extracellular matrix destruction, and joint swelling, to reduce resistance to external force. With the help of the stress load, it can cause cartilage destruction (Choi et al., 2009; Tong et al., 2011). Our study showed that MMP-3 levels were significantly elevated in articular cartilage lesions. As the breaking time was prolonged and pathological damage was aggravated, the serum MMP-3 level gradually increased. It has been suggested that the serum level of MMP-3 reflects the degree of articular cartilage degradation.

Although joint synovial fluid can reflect joint inflammation and cartilage metabolism more sensitively and intuitively than urine and blood, blood monitoring is relatively simple and involves fewer traumas. Furthermore, COMP and MMP-3 have correlation in blood and joint synovial fluid. There was a significant rise in the level of COMP after 2 weeks of breaking. COMP and MMP-3 levels were elevated following the breaking time extension, which shows that COMP and MMP-3 play an important role in maintaining cartilage function. Cartilage damage, metabolism, and repair may all affect COMP and MMP-3 expression. COMP and MMP-3 levels decreased after discontinuing breaking. Measuring serum COMP and MMP-3 levels can be used to predict articular cartilage injury to judge whether OA will occur. OA severity is positively correlated with COMP and MMP-3 levels, which is consistent with the cartilage matrix damage process. Articular cartilage injury was not significant in the early phase of OA, and COMP and MMP-3 level elevation was not obvious. COMP and MMP-3 levels were upregulated with increased cracks, obvious cartilage matrix damage, and increased cartilage cell synthesis, and more residual cartilage appeared. Continuous monitoring of COMP and MMP-3 levels can help identify OA patients with rapid disease progression, and can be used to predict disease progression, which is conducive to OA prognosis.

Long-term joint fixation results in abnormal articular cartilage stress and different degrees of cartilage injury. Sustained abnormal stress on articular cartilage makes the cartilage injury irreversible. Joint breaking excludes joint synovial fluid from the articular cartilage, resulting in a reduction in nutrients and metabolites. It also causes the stagnation of the physiological metabolism of cartilage cells, causing cartilage degeneration and joint dysfunction. There are several theories about articular cartilage degeneration caused by abnormal stress (Honsawek and Chayanupatkul, 2010; Tong, 2011), which are outlined as follows:

1) The cartilage cell damage theory: Cartilage cells have baroreceptors that are subjected to normal stress and stimulate cartilage matrix synthesis and secretion. Breaks cause cartilage stress

changes, resulting in reduced and disordered cell function, decreased cartilage matrix synthesis and secretion, and dysregulated normal replacement. Degenerated cartilage cells synthesize inflammatory cytokines abnormally, leading to over synthesis of matrix metalloproteinases, which further degrades the matrix. Cartilage matrix damage destroys the cartilage cell environment, and further promotes cartilage cell damage, resulting in irreversible cartilage destruction. MMP-3 directly participates in proteoglycan degradation, activates MMP-1, MMP-8, and MMP-9, accelerates collagen pathological degradation, destroys the cartilage matrix, and causes cartilage damage.

2) The biological material fracture theory: Under the action of abnormal stress, articular cartilage causes matrix collagen fiber rupture. Destruction of the network structure that maintains normal cartilage form leads to proteoglycans loss and cartilage cell exposure. Continuous abnormal stress stimulation aggravates matrix damage, damage to the cartilage cell environment, cell function fatigue, and intracellular polysaccharide protein enzyme and collagenase release, leading to cartilage matrix damage. COMP catalyzes type II collagen polymerization by combining with the extracellular matrix, which plays an important role in matrix assembly. The early pathological characteristics of articular cartilage degeneration in OA development are associated with COMP protein redistribution. Cartilage cell synthesis capability decreases when the cartilage matrix damage is severe.

3) The autoimmune response theory: Continuous abnormal stress destroys articular cartilage, leading to specific antigen release that causes an autoimmune reaction. It produces collagen antibodies that aggravate cartilage injury and joint lesions.

This study found that prolonged breaking time led to more severe joint pathological damage. After discontinuing breaking, the joint can repair itself and joint pathological damage is alleviated. This suggests that articular cartilage can restore joint cell function itself before irreversible injury.

To sum up, long-term joint breaking may damage articular cartilage, and COMP and MMP-3 are of great significance in the process of cartilage tissue damage. Recovery time following discontinuation of breaking can promote articular cartilage self-repair. The repair effect is more significant as the recovery time is extended. COMP and MMP-3 levels gradually decreased as articular cartilage repair progressed. Serum and joint fluid COMP and MMP-3 level elevation in the OA model may be associated with a variety of factors, of which cartilage injury is the foremost. COMP and MMP-3 detection can be treated as a means of early OA diagnosis when combined with the observation of clinical characteristics.

Conflicts of interest

The authors declare no conflict of interest.

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