Establishment of a rat model for osteoarthritis resulting from anterior cruciate ligament rupture and its significance

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Received July 31, 2015; Accepted September 11, 2015

DOI: 10.3892/etm.2015.2775

Abstract. The purpose of this study was to examine the establishment of a model concerned with osteoarthritis resulting from the anterior cruciate ligament rupture of rats and investigate the associated mechanism, as well as provide a theoretical basis for clinical treatment of the disease. Forty Sprague-Dawley male rats were randomly divided into two groups of 20 rats each and the anterior cruciate ligament transaction model and knee joint brake model were successfully established. Two rats in the anterior cruciate ligament transection group (10%) and 3 rats in the knee joint brake group (15.0%) died. The survival rate of the two groups was not statistically significant (χ²<0.001, P=1.000). Swelling of the knee joint and synovium of rats in the two experimental groups was aggravated. The Mankin score was significantly higher in the anterior cruciate ligament transection group than that in the experimental group and the difference was statistically significant (P<0.05). By contrast, no significant difference was observed for osteoarthritic severity for the two experimental groups (P>0.05). Analysis of the subgroups showed that the proportion of the anterior cruciate ligament in the experimental group was significantly higher than that of the knee joint brake group, and the difference was statistically significant (P<0.05). By contrast, the difference was not statistically significant in the comparison of the medium and early proportion (P>0.05). The content of protein polysaccharide and II collagen fiber in the experimental group of the anterior cruciate ligament transection was lower than that of the knee joint brake group, and this difference was statistically significant (P<0.05). Thus the mechanism of osteoarthritis may be associated with the decrease in the content of protein and II collagen fibers.

Introduction

Osteoarthritis is a disease that usually occurs in middle-aged individuals and the elderly. It mainly involves the articular cartilage, cartilage bone, synovium, joint capsule and other structures of chronic progressive bone joint disease (1). It is the primary cause of pain and disability for middle-aged individuals and the elderly leading to serious burden to individuals, families and society (2). The etiology and pathogenesis of osteoarthritis remain to be elucidated. Clinical treatment is ineffective. Consequently, the effects are often unsatisfactory. Few studies are available with regard to the repair of articular cartilage of osteoarthritis (3). Thus, the establishment of an animal model of osteoarthritis is an important means to study the pathogenesis, prevention and treatment of osteoarthritis. At present, the osteoarthritis model is induced by the anterior cruciate ligament and knee joint brake experimental models. The establishment and significance of the anterior cruciate ligament model are further discussed in the current study.

Materials and methods

Experimental animals. Forty Sprague-Dawley male rats, 6-8 weeks old and weighing 180-240 g, were provided by the Animal Experimental Center of Shanghai Tongji University. Animals were randomly divided into the anterior cruciate ligament transection group (n=20) and knee joint brake group (n=20). Experimental rats were reared in separate cages and provided with free activities, natural light and ad libitum food. Feeding temperature was 20-23˚C and relative humidity was set at 50-55%. The study was approved by the Ethics Committee of Xuzhou Hospital Affiliated to Jiangsu University, Xuzhou, China.

Establishment of animal model. The specific process for the production of the cross section model of the anterior cruciate ligament (4) was: sodium amytal at a concentration of 30 g/l was used to carry out the abdominal anesthesia (30 mg/kg). A cut was made in the left medial parapatellar to expose the knee joint. The joint capsule was cut to reveal the joint cavity. The patella was laterally dislocated. The knee joint was flexed...
to expose the anterior cruciate ligament as far as possible. The anterior cruciate ligament was disconnected under direct vision with a small sharp knife. The drawer test was carried out to determine the anterior cruciate ligament rupture. The cartilage surface was not damaged during the operation. Following thorough hemostasis, the patella was reset and dissected and then closed layer by layer. For the control group, as a false operation group, the same procedure was used to reach the knee joint by cutting the joint capsule to open joint cavity. The patella was dislocated. The anterior cruciate ligament was exposed prior to the flexion of the knee joint taking care not to disconnect the cruciate ligament, and then the patella was reset and the joint cavity closed. The rats did not need to be fixed after the operation. Intramuscular injection of penicillin at 400,000 units was administered once daily, and continued for 3 days.

The concrete process for the production of knee joint brake model (5) involved medical cotton being placed between the left side of the rat ankle and hip joint. Plaster bandage was evenly fixed to 5-6 layers, followed by fixing of the left knee with extension position of 180 degrees. The control group received no special treatment. Particular attention was paid to the following: i) if the rats bit the plaster, it had to be repaired in a timely manner; ii) pipe type plaster fixation was required to be sufficiently secure in order not to affect the blood circulation of limbs, nor detached. The blood flow to the end of the fixed side of the limb and the plaster casts was closely observed. If the foot became swollen or the plaster casts were removed, the plaster was adjusted or re-fixed in a timely manner.

Specimen sampling and processing. All the animals were sacrificed 8 weeks after cervical dislocation. The knee joint was exposed and observed with the naked eye to observe whether any changes occurred in joint effusion and synovial swelling. Decalcification, dehydration and paraffin embedding of the femoral condyle of the knee joint were performed. The femoral condyle of the knee joint was sectioned and the articular surface of articular cartilage, chondrocytes, mesenchondrium, and tidal line were observed under a light microscope. The score was regarded as the mean value calculated according to the Mankin score by three independent observers. The grading standard was calculated as: a score value of 0-1 was considered as normal cartilage, 2-6 points as early osteoarthrosis, 7-10 points as middle-term osteoarthrosis, and 11-14 points as advanced osteoarthrosis.

The Image-Pro Plus 6.0 image analysis system (Zesir, China) was used to capture images of each specimen at three different views and the average absorbance value of positive staining for the unit area was measured by 10x40 high power lenses (Zesir, China). Semi-quantitative analysis was used to express the content of the protein in the cell matrix. Collagen type II polyclonal antibody and the immunohistochemical staining SP kit was purchased from Wuhan Boster, Bio-Engineering Co., Ltd., Wuhan, China. PBS replaced the primary antibody to serve as the negative control. The experimental procedures of immunohistochemistry were performed according to the manufacturer's instructions. The Image-Pro Plus 6 image analysis system was used to capture images as mentioned above. Semi-quantitative analysis was used to express the content of collagen type II fibers in the cell matrix.

Statistical analysis. Data were statistically analyzed using IBM SPSS 20.0 software (Hampshire, UK). The measurement data were presented as the mean ± standard deviation. The independent t-test was used for comparison between groups. Enumeration data were expressed as a percentage, and the χ² test was used for comparisons between groups. P<0.05 was considered to indicate statistically significant results.

Results

Visual observations. Two rats (10%) died in the anterior cruciate ligament transection group with 18 rats remaining alive and 3 rats (15.0%) died in the knee joint brake group with 17 rats remaining alive. The survival rate of the two groups was not statistically significant (χ²<0.001, P=1.000). In the control group, the synovial membrane was generally normal and the surface of the articular cartilage was light blue and white as well as smooth and bright with an integrated surface. Swelling of the knee joint and synovium of the rats in the experimental group was aggravated. Swelling and the weakening of the gloss of the cartilage surface was more severe in the anterior cruciate ligament transection group compared to the knee joint brake group.

Mankin score of articular cartilage. The difference in Mankin score of the two groups compared to the control group was not statistically significant (P>0.05). The difference between the two experimental groups and the control group following evaluation of the scores was statistically significant (P<0.05). The score of the anterior cruciate ligament transection group (12.7±3.4) was significantly higher than that of the knee joint brake group (9.8±2.2), and the difference was statistically significant (P<0.05). However, analysis of the sub-groups showed that the proportion of the anterior cruciate ligament in the experimental group (66.7%) was significantly higher than that of the knee joint brake group (47.1), and the difference was statistically significant (P<0.05). The difference in the comparison of the medium and early proportion was not statistically significant (P>0.05) (Table I).

Comparison of the content of protein polysaccharide and II collagen fibers. No significant difference was identified for the contents of protein and collagen fibers between the two groups (P>0.05). The contents of the two experimental groups were significantly decreased compared with those of the control group, and the difference was statistically significant (P<0.05). Furthermore, the content of the protein polysaccharide (0.26±0.01) and II collagen fiber (0.26±0.02) in the anterior cruciate ligament experimental group was significantly lower than that in the knee joint brake group (0.35±0.04 and 0.37±0.03, respectively) and the difference was statistically significant (P<0.05) (Table II).

Discussion

Comparison of two models. Instability of the joint is caused when the anterior cruciate ligament is disconnected. Alteration of the mechanical environment may result in the occurrence of knee osteoarthritis. Experimental evidence has shown that cutting the anterior cruciate ligament of the knee joint of dog...
may result in the developmental process of the experimental limb. The finding is consistent with that of the natural occurrence (results from anterior cruciate ligament rupture), and its final pathological features are similar to those of osteoarthritis (6). Stoop et al (7) disconnected the anterior cruciate ligament from the knee joint of Wistar rats. The animals were then sacrificed on the 2nd, 7th, 14th, 28th and 70th day, to detect collagen type II. Results of the study by Stoop et al (7) showed that the earliest changes in cartilage damage following the anterior cruciate ligament transection occurred in the cartilage cells and the cartilage matrix of the surface zone. Swelling in the surface layer of the cartilage cells and the surface fibrillation appeared 14 days after the operation and these changes became more obvious after 4 and 10 weeks of surgery. Therefore, it seems that the articular surface of early mechanical overloading induces the degeneration of cartilage (8). Additionally, articular cartilage degeneration is associated with the local expression of the degradation product of collagen type II, and collagenase plays an important role in the degeneration of articular cartilage (8).

Joint braking is the most common way to induce the model. In the case of non-injury of the joint stability, the degeneration of articular cartilage in rats is induced by joint immobilization (9). The joints of the animals are fixed in a certain position over a long time period, restricting the movement of the joints and the contraction of muscle and joint capsule, thereby creating excessive stress on the joint surface. The muscle contraction across the knee is reduced, leading to the atrophic changes of the articular cartilage, which can lead to the occurrence of osteoarthritis (10-11).

The two methods successfully constitute a knee joint degeneration model and can reflect the pathological process of articular cartilage degeneration of osteoarthritis in a comparatively comprehensive manner, although there are some differences between the two methods inducing the osteoarthritis model. Visual observation revealed that the anterior cruciate ligament transection results in a greater swelling of the knee joint, and a lighter color of the joint surface. Additionally, the Mankin score of the experimental group was significantly higher than that of the knee joint. Although the comparison concerned with the severity of osteoarthritis in the experimental group was not statistically significant, results of the subgroup analysis revealed that the advanced proportion was significantly higher than that of the knee joint immobilization group. Furthermore, the content of the protein polysaccharide and II collagen fiber was lower compared to the knee joint brake group. These results indicated that the two methedns can successfully produce the joint degeneration model, which

### Table I. Mankin score of articular cartilage.

| Group                                      | Cases | Control group score | Experimental group score |
|--------------------------------------------|-------|---------------------|--------------------------|
| Anterior cruciate ligament transection group, n (%) | 18    | 0.9±0.4             | 12.7±3.4                 |
| Knee joint brake group, n (%)              | 17    | 0.8±0.2             | 9.8±2.2                  |
| t                                          |       | 0.529               | 5.247                    |
| P-value                                    |       | 0.417               | 0.024                    |

### Table II. Comparison of the content of collagen and II collagen fibers.

| Group                                      | Protein polysaccharide | Collagen type II |
|--------------------------------------------|------------------------|-----------------|
|                                            | Control group          | Experimental group |
|                                            | Control group          | Experimental group |
|                                            |                        |                  |
| Anterior cruciate ligament transection group | 0.55±0.03              | 0.26±0.01        |
| Knee joint brake group                      | 0.64±0.02              | 0.41±0.02        |
| t                                          | 0.528                  | 0.37±0.03        |
| P-value                                    | 0.267                  | 0.639            |
can reflect the pathological process of the articular cartilage degeneration, although there are differences between the two methods of the osteoarthritis model (12).

Significance of model establishment. Osteoarthritis is the interactive effect of mechanical and biological factors, resulting in loss of balance in the synthesis and degradation of articular cartilage cells, extracellular matrix and cartilage degradation (13). Collagen and protein polysaccharides are the main components of the extracellular matrix of articular cartilage and the decisive factors of the surface tension of cartilage (14). In normal cartilage, there is a large amount of collagen fibers synthesized by chondrocytes (mainly collagen type II) (15). Protein polysaccharides and other substances are secreted in the extracellular matrix, constituting the outer fiber grid, which supports and protects cartilage cells. The two are complementary to each other (16). Collagen is an important component of the extracellular matrix component. The quantitative and qualitative changes of collagen type II are the direct cause of the loss of normal articular cartilage biomechanics. The change is closely associated with bone osteoarthritis. The change in collagen type II is the direct cause of the loss of articular cartilage and its normal biomechanical properties (17).

In summary, the mechanism of osteoarthritis may be associated with the decrease of the content of protein and II collagen fibers. Two types of models have been described in the present study, both of which yielded reasonable results, albeit, the cross ligament rupture model appears to be the more optimal model as compared to visual observance, the Mankin score comparison, bone arthritis severity and the molecular level and the content of collagen fiber II.

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