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Original Article

Characterization of pathological and biochemical changes in rat destabilization of medial meniscus models of osteoarthritis

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Abstract: Destabilization of medial meniscus (DMM) surgical instability models of osteoarthritis (OA) have been underutilized despite having many inherent advantages. At present, there is a lack of studies clarifying cartilage degenerative changes induced by DMM in rats, which would further verify a greater use of this model. Therefore, the present study compared biochemical and histological characteristics in rat models using DMM against the anterior cruciate ligament transection (ACLT). OA was surgically induced in male SD rats by transection of the medial meniscotibial ligament (MMTL) or anterior cruciate ligament in the right femorotibial joint. Histopathological and biochemical (glycosaminoglycans (GAG) content) evaluations were performed at the end of the 2nd, 4th, and 6th week after OA induction. The ACLT model gave severe OA and, in some cases, severe subchondral erosion of the tibial plateau. Surgical DMM was less invasive than the ACLT procedure. Lesions progressed from mild-to-moderate OA at 4 weeks to moderate-to-severe OA at 6 weeks post-surgery. Destruction of the subchondral bone was not observed in the early DMM model, while detectable reduction in GAG content in the DMM group was observed (DMM: 4 ± 0.62; normal: 7 ± 0.51) as early as 4 weeks post-surgery. In conclusion, the DMM model produced more moderate degenerative changes than ACLT, with the advantages of being minimally invasive and reproducible. Therefore, more extensive application of DMM in rats as an animal OA model should be promoted.

Keywords: Osteoarthritis, animal models, rat, cartilage degeneration, instability, pathology

Introduction

Osteoarthritis is a chronic joint disease, mainly affecting articular cartilage, characterized by progressive cartilage degeneration, subchondal bone changes, osteophyte formation, and low-grade synovitis [1-3]. It is, by far, the most common form of joint disorders. In 2004, OA was estimated to cost the US $336 billion [4]. Development of OA is hard to study clearly in humans. Progression of OA is usually slow and highly subject to various individual factors, such as occupation, lifestyle, hormonal status, and body mass index [5].

Animal models of OA are a significant substitution, not only providing a method to study OA pathology, but also promoting the development of OA diagnostics and therapeutic drug development [6-8]. Surgical instability models are the most commonly used OA models [3]. Of the various kinds of surgical methods, the surgically induced destabilization of medial meniscus (DMM) model has been proven to be closer to human OA than other methods, such as the anterior cruciate ligament transection (ACLT) model of OA [8-10]. However, articular cartilage is very thin in mice, lacking discernible radial, transitional, and superficial layers. Furthermore, postoperative management of mice is difficult [11]. Therefore, there is a lack of studies clarifying cartilage degenerative changes induced by DMM in appropriate animal models.

Compared to mice, rats are inexpensive and easy to care for. More importantly, the articular cartilage in rats is thick enough to induce both

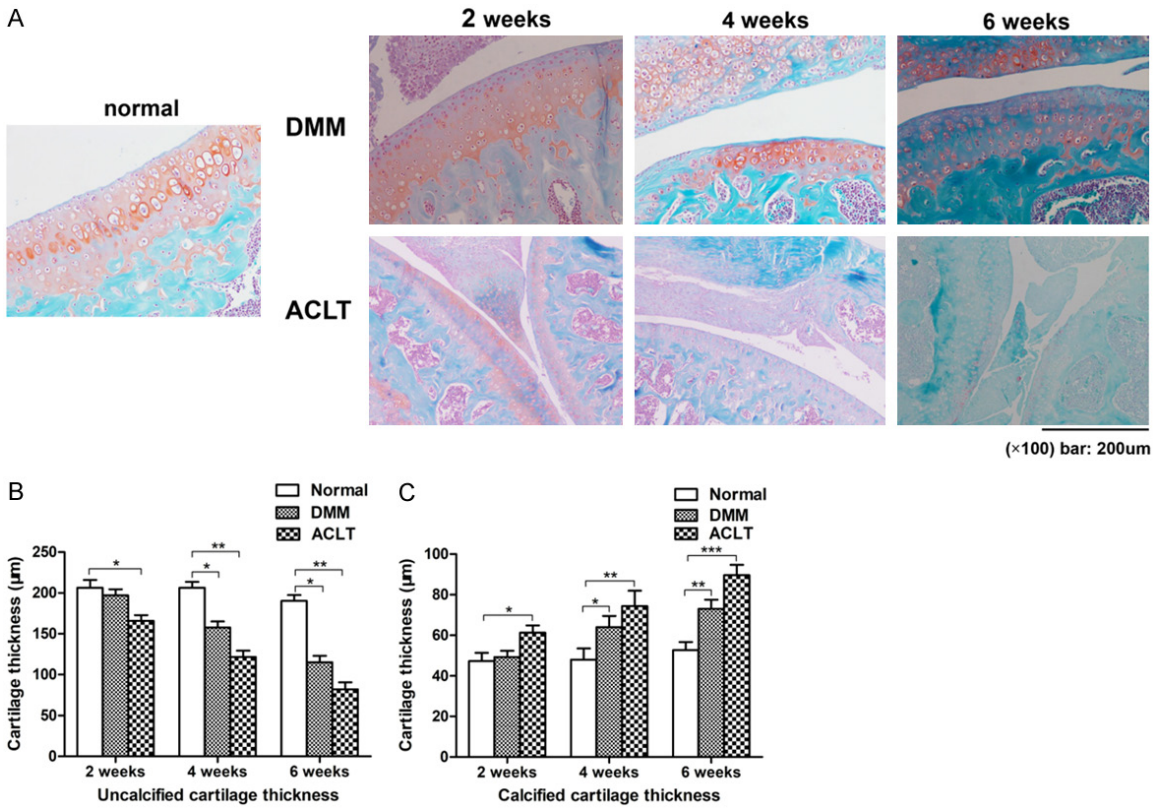

Figure 1. Histological analyses of knee joint changes and quantitative assessment of cartilage thickness in rat DMM and ACLT models of osteoarthritis. A. Sagittal section through the medial tibial condyles of rat (100X). Normal articular cartilage structures showing smooth articular surface, normal chondrocytes with columnar orientation, intact tide mark, and subchondral bone. At 4-6 weeks post DMM and ACLT, a confined area of cartilage destruction(arrow) was observed as reflected by the irregularity of the articular surface and moderately reduced chondrocyte numbers. The tidemark remained visible and the sub-chondral bone remained intact. B and C. Decreases in uncalcified cartilage thickness and increases in calcified cartilage thickness were observed in DMM and ACLT joints, compared with the normal joints. Relative to control: $^{*}P < 0.05$; $^{**}P < 0.01$, $^{***}P < 0.001$.

partial and full-thickness cartilage defects [7]. This advantage has allowed rats to be used for the study of cartilage restoration and reconstruction techniques. However, pathological and biochemical changes of cartilage degeneration in rat DMM models of OA have not been comprehensively characterized. The present study examined destabilization of medial meniscus (DMM) models in rats, observed the disease development process in this model for up to 6 weeks post-surgery, and examined sequential pathological changes in articular cartilage and other structures of the knee joint.

Material and methods

Animal care

All experiments were approved by the Institutional Animal Care and Use Committee of Shangdong University (Jinan, China) and Wuhan University (Wuhan, China) and all animal procedures were performed in strict accordance with institutional and national guidelines. A total of 30 male Sprague-Dawley rats from the Experimental Animal Center of Wuhan Medical University were used for the experiment. The average weight of the rats was 230 ± 16 g at the time of the experiment. Four rats were raised in a cage and were allowed free movement and free access to food and water.

Surgically induced OA (DMM)

The rats were divided into two groups: Group 1 (n = 15) underwent the ACLT procedure and group 2 (n = 15) underwent the DMM procedure. In the ACLT group, the ACL was transected with a micro-surgical knife under direct visualization. Destabilization of the medial me-

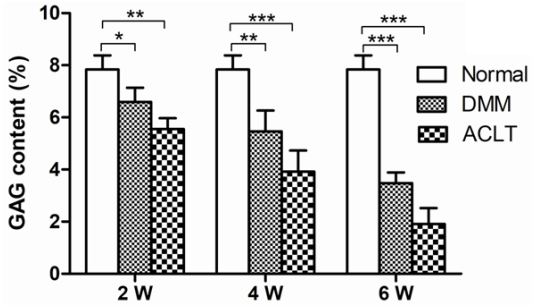

Figure 2. GAGs content of articular cartilage in Normal, DMM, and ACLT joints. GAGs content was lower in DMM and ACLT groups at the tibial plateau after 4 weeks post surgery and consistent with histological analysis. All results represent mean ± S.D. for three times independent experiments. Relative to control: $^{*}P < 0.05$; $^{**}P < 0.01$, $^{***}P < 0.001$.

niscus (DMM) surgery was performed, according to established methods [7]. Briefly, rats were anesthetized with 2-3% isoflurane and adjusted to maintain a surgical plane of anesthesia. The right knee joint was opened along the medial border of the patellar ligament and the medial meniscotibial ligament (MMTL) was dissected to destabilize the medial meniscus. Contralateral knee joints of the same rats were not subjected to any intervention and were used as controls. OA was, however.

Histological preparation and assessment

Rats were euthanized at 2, 4 and 6 weeks post-operatively. All stifle joints (n = 60 joints from 30 rats) were fixed in 4% paraformaldehyde for 24 hours and decalcified in 10% ethylenediamine tetraacetic acid (EDTA) for 7 days on a shaker. They were processed, embedded intact into paraffin, and 5 μm sagittal sections were taken through the entire joint at 60 μm intervals. Two to four sections were stained with Safranin-O for evaluation. Each knee yielded 10-15 slides for scoring by three blinded observers using modified Mankin scoring system [12].

Biochemical analysis

Glycosaminoglycan (GAGs) levels were determined using Blyscan sulfated Glycosaminoglycan assay kit (Biocolor Ltd., UK), according to manufacturer protocol. Spectrophotometric absorbance measurement for GAG was obtained at 656 nm wavelength. Levels of GAGs and cartilage wet mass were measured in the dissected articular cartilage of the total surface of the femur and tibia without any processing.

Statistical analysis

Results are expressed as mean ± standard deviation for each parameter examined. All statistical calculations were performed using GraphPad Prism (GraphPad Prism version 5.0 for Windows, GraphPad Software, San Diego, CA). Statistical comparisons between various groups in this study were performed with two-way analysis of variance. Values of $P < 0.05$ indicate statistical significance.

Results

The animals recovered about 15 minutes after surgery. No infections developed during the post-operative period. Body weights between ACLT and DMM rats were without significant differences. Histopathological changes in the DMM model appeared sequentially and time dependent. The medial tibial plateau developed more obvious and reproducible pathological changes of OA than the lateral tibial plateau in the DMM models of OA, consistent with previous studies in a mice model [7]. Articular cartilage surfaces appeared to be smooth in the knee joints, up to 2 weeks post-surgery (**Figure 1A**). Focal cartilage thickness in DMM models was not different from that in the sham group. However, there was detectable cartilage degeneration (decrease of PG) at 2 weeks post-surgery. After 4 weeks, articular surface fibrillation was further extended (**Figure 1A**). Compared to the ACLT group (**Figure 1A**), however, there was no osteophyte formation at 6 weeks post-surgery. During OA progression, thinning of cartilage occurred from all directions, characterized by surface degradation and the increase of calcified cartilage layers or tide rising. The average thickness of cartilage (uncalcified layers and calcified layers) were calculated by histomorphometric analysis. In the DMM OA model, **Figure 1B** demonstrates the cartilage loss at 4 weeks post-surgery in the medial tibial plateau. Uncalcified layer thickness significantly decreased while the thickness of calcified cartilage slowly increased.

According to biochemical analysis, **Figure 2** represents GAGs content of articular cartilage

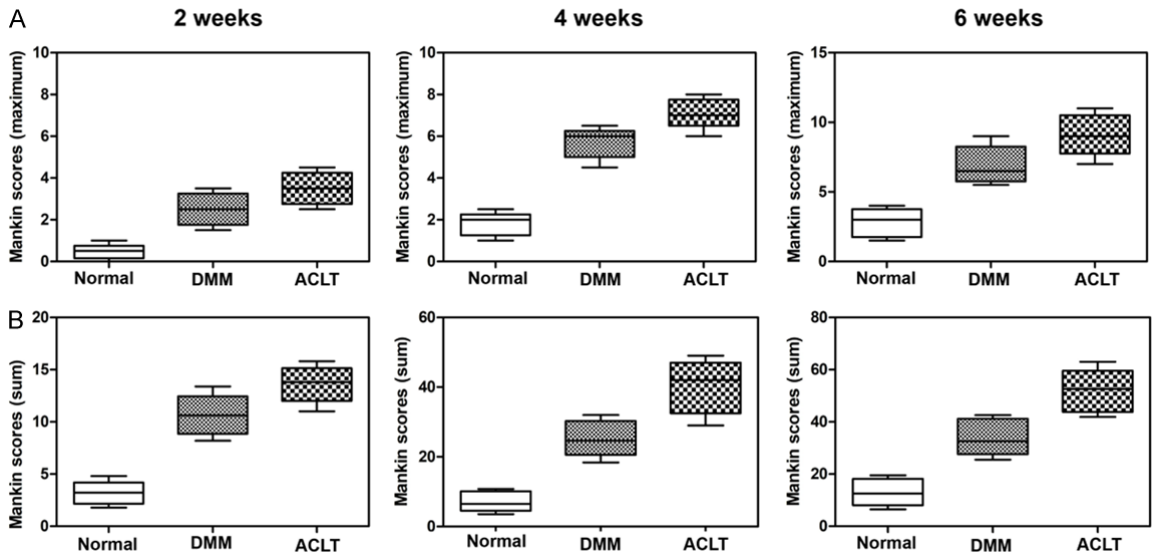

Figure 3. Modified Mankin scoring of medial tibial plateau for DMM and ACLT models and box and whisker plots showing the central horizontal line the Mankin score. (A) Mean maximum histologic scores and (B) mean summed scores. The data range is represented by whiskers.

for ACLT and DMM groups. Results showed that GAGs content was significantly lower in articular cartilage in the surgical group, compared to the normal group ($P < 0.05$) from 2 weeks after surgery, indicating that ACLT and DMM had reached its maximal damaging effects as early as post-surgery, persisting for 2 weeks. However, in the DMM group, GAGs content was significantly lower at 4 weeks compared to 2 weeks, indicating that considerable damage to the cartilage may have occurred at a relatively slower pace when OA was induced by this method.

Modified Mankin scores throughout the progression of the experiment are shown in **Figure 3**. At 2 weeks in the DMM group, there was a mild increase of maximum scores (3.7 ± 0.5, $P < 0.05$, **Figure 3A**) and summed scores (11.9 ± 1.2, **Figure 3B**). At 4 weeks, maximum scores (5.7 ± 0.75, $P < 0.05$, **Figure 3A**) and summed scores (25.2 ± 5.2, **Figure 3B**) are shown. From 4 to 6 weeks, these indicators increased over time, representing a progression from mild-to-moderate to moderate OA in the DMM model. More severe OA was observed in the ACLT group at 4 weeks, according to maximum scores (7.1 ± 0.1, $P < 0.01$, **Figure 3A**) and summed scores of (40.2 ± 7.8, **Figure 3B**). On the other hand, as a control, in the normal group, results showed negligible OA with maximum scores of 1.2 ± 0.3 and summed scores of 3.6 ± 1.1 at 2 weeks, along with mildly elevated maximum scores of 3.1 ± 0.2 and summed scores of 11.9 ± 1.5 at 6 weeks (**Figure 3B**). More importantly, the present study found that Mankin scores were increased locally in the middle region over time in the DMM group, consistent with pathologic features of human OA.

Discussion

It is very important to investigate OA pathophysiology using a reliable OA model for OA diagnosis and assessment of disease modifying OA drug efficacy. Using a DMM OA model in rats, the present study successfully demonstrated that osteoarthritic pathology changes, including cartilage degradation, chronic-inflammation, and reconstruction of subchondral bones, developed sequentially and progressively with time. Initial osteoarthritic changes in the DMM OA model in rats were found in the uncalcified layer of articular cartilage, including loss of PG, decreased chondrocyte cell number, and focal fibrillation of the articular surface. In middle and advanced stages of OA (4 and 6 weeks post-surgery), significant articular cartilage thinning and PG loss, induced by joint instability, were found, consistent with previous studies using ACLT and meniscus resection models in rats [13, 14].

Destabilization of medial meniscus (DMM) OA models were first used in mice in 2007, con-

cluded more reliable than other surgery methods. For example, anterior cruciate ligament transection (ACLT) induced OA has been progressing too fast and considered to be too severe to represent human OA [15]. In contrast, the DMM induced OA model has provided exceeding reproducibility and more slow progression, as with human OA. It is the optimization method of choice for assessing OA models. Furthermore, this method was also successfully used for efficacy evaluation of OA treatment in mice. However, articular cartilage in mouse knees is very thin, thus it is absent of discernible radial, transitional, and superficial layers. Moreover, postoperative management in mice is very difficult. Compared with mice, rats are not only inexpensive but also easy to care for. More importantly, the articular cartilage is thicker than mice and is thick enough to induce both partial and full-thickness cartilage defects. On the other hand, the pathological changes described in rat OA models are closely comparable with surgically induced models in dogs or rabbits. While significant cartilage loss and osteophyte formation take more than a year to develop in ACLT dogs, diseases progress at a faster rate in rat models. Compared to large animals, besides the advantages of relatively low costs and small size, present results suggest that the use of rat DMM OA models will significantly shorten observation times during drug intervention studies [16].

In this study, mild to moderate cartilage injuries were present, mostly on the central weight bearing area of tibial plateau, and degeneration aggravated through the progression of OA. Interestingly, damage to posterior tibial plateaus was not observed in the rat DMM model, as with human OA. To eliminate the operation directly leading to cartilage damage, rats were euthanized immediately after surgery. No cartilage injuries were observed, supporting the hypothesis that cartilage injuries are caused by changes in joint mechanical stability after DMM.

In articular cartilage, chondrocytes constitute only 4% of its wet weight, while water occupies 65-85% within a network of collagen fibrils (15-20%) and proteoglycan aggregates (3-10%) [17]. Glycosaminoglycans (GAGs), the main components of PG in articular cartilage, are negatively charged due to ionized sulfate and carboxyl groups. They draw water through the collagen fibril and are essential factors to the structure and biomechanical properties of cartilage [18]. It has been widely accepted that the loss of GAGs in articular cartilage is an important early event in OA progression [19]. In this study, levels of GAGs content in articular cartilage were measured, successfully reflecting the biochemical changes of cartilage related to the degenerative process in DMM induced OA. Compared to normal knee joints, DMM induced osteoarthritis rat models showed obvious differences in cartilage structure (histopathology) and GAG content, as early as 2 weeks post-surgery. It has been suggested that in early stages of OA, the GAG content has been decreased, consistent with previous studies [20]. In addition, this study also observed that the content of GAGs decreased gradually with the development of OA. The main limitation of the present study was the short experimental period. only 2, 4 and 6 weeks after surgery. Thus, long-term changes in cartilage and subchondral bone are unknown.

In conclusion, the results of this present study demonstrate that destabilization of medial meniscus induced OA in rat knee joints effectively induces degenerative changes in articular cartilage. The loss of mechanical stability results in articular cartilage degeneration and subchondral bone defects in the early phase site, specifically in the middle region of knee joints. Moreover, the present results indicate that the area of cartilage covered by menisci is easily degraded, resulting in early DMM surgical induced OA. Given its minimal invasiveness and convenience, wide use of DMM in rats as a translational OA model should be considered.

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Disclosure of conflict of interest

None.

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