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Assessment of clinical symptoms and pathological effects of degenerative arthritis in rats

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Abstract

Degenerative arthritis, particularly affecting the knee joint, is a prevalent condition in Taiwan. While milder cases may only result in slight discomfort during walking, severe cases can lead to significant mobility issues and even loss of basic movement capabilities. Unfortunately, once articular cartilage is lost due to degenerative arthritis, it cannot be regenerated. Currently, the severity of knee joint degeneration is classified into primary, intermediate, and severe stages, which guides the selection of treatment options. These treatments range from non-surgical interventions to surgical procedures. Given the significance of preventing knee joint degeneration, researchers have been exploring various methods. One such approach involves establishing animal models to mimic degenerative arthritis conditions in humans. In this study, researchers utilized anterior cruciate ligament transection (ACLT) combined with medial meniscectomy (MMx) surgery to create a rat model of degenerative arthritis. The successful establishment of this model provides a valuable tool and assessment of clinical symptoms and pathological effects of degenerative arthritis in rats for studying potential drugs and designing therapeutic strategies for degenerative arthritis. By leveraging this rat model, researchers can investigate the efficacy of different pharmaceutical interventions and therapeutic approaches. This could ultimately lead to the development of novel treatments for degenerative arthritis, benefiting individuals suffering from this debilitating condition.

Keywords: Anterior cruciate ligament transection; Degenerative arthritis; Medial meniscectomy; Osteoarthritis; Rat model; Surgery induction

1. Introduction

Osteoarthritis (OA), commonly known as degenerative arthritis, presents a significant health concern for individuals in Taiwan, particularly affecting the knee joints. This condition is characterized by pain, especially during weight-bearing activities. While mild cases may only result in discomfort while walking, severe OA can severely impact mobility. Given the substantial weight-bearing role of the knee joints, the wear and tear on the articular cartilage are inevitable, and unfortunately, cartilage loss in OA is irreversible. Therefore, prevention strategies are crucial [1-4].

Many OA patients seek dietary advice to maintain joint health, but effective prevention primarily involves reducing knee joint burden. This can be achieved through weight control, minimizing weight-bearing activities, and avoiding excessive exercise. While various factors contribute to cartilage degradation, reducing knee joint burden remains a key preventive

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measure [4-5]. The severity of knee joint degeneration is categorized into primary, intermediate, and severe stages, with treatment options ranging from non-surgical interventions to surgical procedures. However, it's important to note that while treatments aim to alleviate symptoms, they cannot reverse degeneration. Treatment approaches include anti-inflammatory drugs and rest for primary stage OA, corrective surgery or partial knee replacement for mid-stage OA, and total knee arthroplasty (TKA) for severe cases. TKA procedures are increasingly common in Taiwan, especially among the elderly population [6-8].

Advancements in research and medical technology have led to more diverse treatment options. Tailored surgical treatments, such as proximal osteotomy corrective surgery, aim to alleviate OA symptoms without resorting to joint replacement. Additionally, the approval of cell therapy by the Taiwanese government offers potential for cartilage regeneration, though success rates vary. Despite these advancements, the development of an optimal OA animal model remains crucial for drug research and therapeutic strategy development. While artificial joint replacement is effective, exploring alternative treatments before surgery is essential. Collaboration between healthcare professionals, patients, and their families is vital for devising effective treatment plans [9-10]. In conclusion, while artificial joint replacement remains a reliable treatment option, ongoing research endeavors aim to enhance OA treatment outcomes. The development of an ideal animal model is imperative for advancing drug research and therapeutic strategies in OA management.

2. Materials and Methods

2.1. Experimental Animals

Adult male Sprague Dawley (SD) rats, 18 in total and 9 weeks old, were procured from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan) under specific pathogen-free conditions. These rats were maintained on a standard laboratory diet (No. 5001, LabDiet®; PMI Nutrition International, St. Louis, MO, USA) and provided with distilled water ad libitum throughout the experimental period. The animals were housed in an environment with a room temperature of 24-27°C, humidity ranging from 60% to 70%, and a photoperiod consisting of 12 hours of light and 12 hours of darkness. Following a one-week acclimation period, the study commenced. All animal experiments were conducted in accordance with the guidelines outlined in protocol IACUC-112049, which was approved by the Institutional Animal Care and Use Committee (IACUC) of the Agricultural Technology Research Institute.

2.2. Experimental Design

The 18 SD rats were used in this study. The surgical induction for OA was performed. All rats' body weight (BW) change rate (%), the widths of the knee joints, food intake, and water intake were measured after the surgery. At the end of the experiment (12^{th} week), the knee joint section confirmed the progressive deterioration of OA joint with the grade (severity of lesion) and stage (area of lesion) of the tibial and femoral articular surface.

2.3. Anterior Cruciate Ligament Transection Combined with Medial Meniscectomy-induced OA Rat Model

Surgery-induced OA rat model was proceeded as described previously [11]. The anterior cruciate ligament transection (ACLT)-medial meniscectomy (MMx) surgery was performed on the left knee joint of rats by a senior veterinarian. Briefly, male SD rats (270-280 g BW, 9 weeks old) were anesthetized with 5% isoflurane and then maintained with 2% isoflurane. Hair was removed around the left knee skin and sterilized with povidone-iodine solution and 70% ethanol. Later, the left knee joint skin was cut with a sterilized scalpel. An incision was made in the medial aspect of the left joint capsule. The anterior cruciate ligament was transected using a sterilized scalpel, and the medial meniscus was removed completely using a sterilized tenotomy scissor. Following ACLT-MMx surgery, the incision joint was irrigated with normal saline/antibiotics. The incision joint capsule was sutured with 4-0 vicryl. The 4-0 monofilament nylon was used for the incision skin closure. Finally, the wound area was sterilized with povidone-iodine solution. Analgesics (3 mg/kg/day ketoprofen) and antibiotics (30 mg/kg/day amoxicillin) were administered intramuscularly for 3 days to prevent pain and infection.

2.4. Measurement Values of Physiological Items

All rats were recorded the changes in BW during the experiment. In addition, the water intake and food intake in rats were also recorded daily.

2.5. Measurement of the Width of Knee Joints

The width of the knee joint was measured with an electronic digital caliper (Mitutoyo America Corporation) once a week after ACLT-MMx surgery until the end of the experiment. The level of actual joint swelling induced by ACLT-MMx surgery was evaluated according the width (mm) of the surgical right knee joints of OA rats minus the width (mm) of the left knee joints of OA rats.

2.6. Animal Specimen Collection and Examination

The experiment lasted for 12 weeks. At the end of the experiment, the experimental animals were euthanized using CO_2, and blood was collected via cardiac puncture to obtain serum for subsequent analysis. The tibia of all rats were dissected to the femur level, and the lengths of the tibiae and femurs were measured. After measurement, the tibia and femurs were immersed in 10% neutral buffered formalin for subsequent sectioning, staining, and pathological examination. The tibia and femurs of the rats were fixed in formalin for 1 week, followed by decalcification (Surgipath Decalcifier II). After decalcification, standard dehydration procedures were followed, and the specimens were embedded in paraffin blocks. Longitudinal sections were then made and stained with H&E. Following staining, the sections were observed under a light microscope using software for quantitative analysis. Specific regions near the growth plate were selected for measurement of bone tissue morphology parameters, following the criteria used by Bitto et al. [22] and Lin et al. [26] for scoring trabecular bone structure and trabecular bone quality.

2.7. Statistical Analysis

All values are presented as mean ± SD or mean ± SEM. One-way analysis of variance was conducted using Graphpad Prism 6 statistical software to analyze differences between different treatments. A significance level of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Measurement of BW Change Rate, Water Intake, and Food Intake of Rats

During the experiment, the changes in BW, food intake, and water intake of rats are shown in Table 1. During the entire experimental period, the rats showed that the ACLT-MMx surgery would slow the growth of the rats, but the BW change rate (%) of rats maintained a steady increase with time (Table 1). In addition, the average daily food intake and water intake of rats were normal status during the experimental period (Table 1).

Table 1 During the experiment, the changes in body weight (%), water intake (mL), and food intake (g) of rats (n = 18)

Experimental time	BW change rate (%)	Daily water intake (mL)	Daily food intake (g)
1st week	5.22 ± 2.33	38.19 ± 5.06	28.39 ± 1.76
2nd week	11.43 ± 3.40	40.01 ± 6.80	28.60 ± 2.16
3rd week	19.51 ± 4.14	35.43 ± 4.20	28.52 ± 1.86
4th week	26.38 ± 5.95	42.09 ± 5.87	27.88 ± 1.49
5th week	28.66 ± 5.62	45.35 ± 5.47	25.94 ± 1.43
6th week	32.02 ± 6.30	41.97 ± 5.38	27.08 ± 1.73
7th week	36.33 ± 7.01	41.64 ± 6.04	27.83 ± 1.89
8th week	40.17 ± 7.51	44.04 ± 4.84	28.10 ± 1.86
9th week	44.27 ± 9.05	37.57 ± 4.01	28.41 ± 0.52
10th week	45.81 ± 8.77	35.70 ± 4.32	27.74 ± 0.96
11th week	47.83 ± 8.99	37.52 ± 5.19	28.43 ± 1.86
12th week	51.12 ± 9.61	38.25 ± 7.98	27.75 ± 2.12

Footnote: 1. The body weight (BW) of rats was measured weekly. BW change rate (%) was calculated using the formula: [(BW of the week - initial BW) / initial BW) × 100%. All values were presented as mean ± SEM; 2. The water intake (mL) and food intake (g) of rats were measured weekly. The average daily water intake (mL) and food intake (g) was calculated using the formula: total weekly water intake (mL) and food intake (g) / the number of rats per cage / 7 days. All values were presented as mean ± SD.

3.2. Measurement of the Width of Knee Joints of Rats and Calculation of the Ratio of Knee Joint Width between the Left and Right Knee Joints

Using of an electronic digital caliper to measure the width of the knee joint of the rat's hind limbs and measure it once a week to understand the degree of swelling of the rat's knee joint. As shown in Figure 1, a significant increase in the rat's right knee joint swelling was observed after ACLT-MMx surgery. Under the gross examination, the rats' right knee joints were wider than the left knee joints.

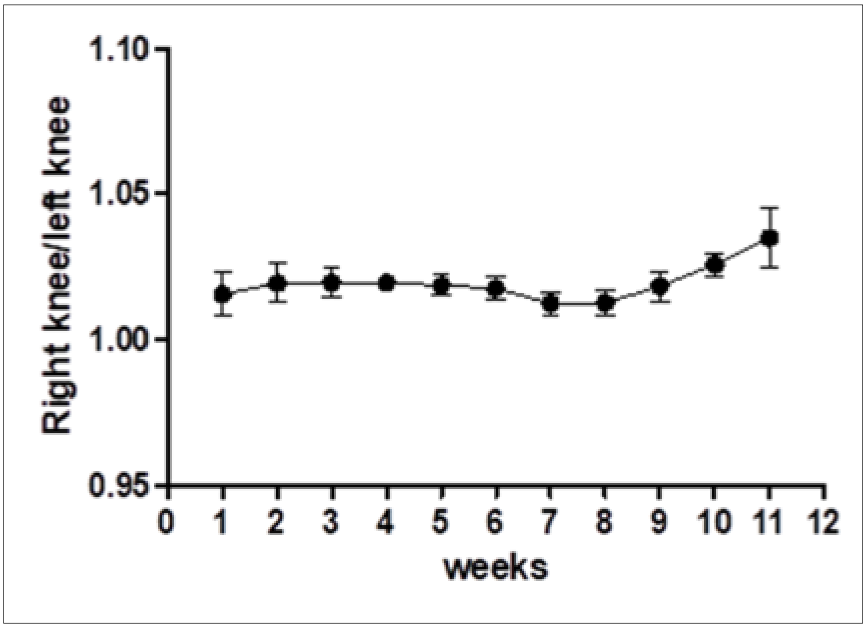

Figure 1 Weekly measurement of the width of knee joints of rats and calculation of the ratio of knee joint width between the left and right knee joints. The values (the width of right knee joint / the width of left knee joint) were presented as mean ± SEM

3.3. Pathologic Examination of Rats' Knee Joints

Longitudinal sections of the tibia and femurs of rats were prepared and subjected to trimming, followed by staining with H&E. After staining, specific areas near the growth plate of bone were selected for the measurement of histomorphometric parameters under a light microscope using quantifiable software. The results revealed the grade (severity of lesion) and stage (area of lesion) of right tibial and femoral articular surface (Figure 2) were evaluated and calculated OA score (OA score = grade score × stage score; average of both ends of the joint surface is the sum of the right tibial and femoral OA scores divided by 2). For the right tibial articular surface, the OA lesion score at 1 month post-operation was 5.33 ± 0.94, at 2 months post-operation was 11.33 ± 6.18, and at 3 months post-operation was13.90 ± 7.69. For the right femoral articular surface, the OA lesion score at 1 month post-operation was 5.0 ± 4.97, at 2 months post-operation was 6.67 ± 4.11, and at 3 months post-operation was13.30 ± 6.51. Regarding the lesion scores of both ends of the right knee joint surfaces, the average OA score at 1 month post-operation was 5.17 ± 2.78, at 2 months post-operation was 9.33 ± 4.78, and at 3 months post-operation was13.6 ± 6.68 (Table 2).

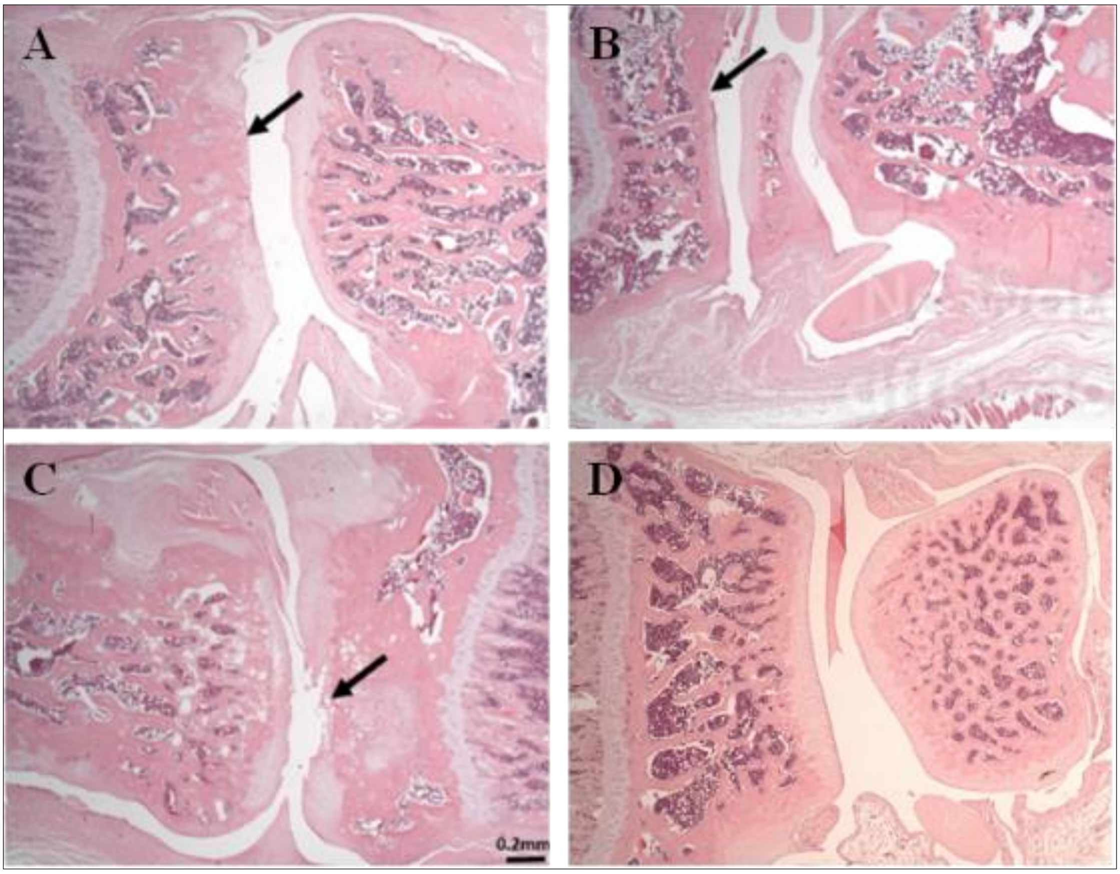

Figure 2 The histological changes in the knee joints of rats with degenerative arthritis. The black arrows indicate the lesion areas of articular cartilage. (A) 4 weeks post-surgery; (B) 8 weeks post-surgery; (C) 12 weeks post-surgery; (D) rat's normal joints. H&E stain, magnification 40×, and bar scale was 0.2 mm

Table 2 Severity of pathological changes in the tibial and femoral articular surface of the knee joint at different time points in operated rats. (A) Tibial articular surface score; (B) Femoral articular surface score

	4W	**8W**	**12W**
Articular surface of tibia			
grade	3.67 ± 1.70	3.67 ± 1.25	4.50 ± 1.43
stage	1.67 ± 0.47	3.00 ± 0.82	2.90 ± 1.04
OA score	5.33 ± 0.94	11.33 ± 6.18	13.90 ± 7.69
Articular surface of femur			
grade	2.33 ± 1.25	3.67 ± 1.70	4.30 ± 1.27
stage	1.67 ± 0.94	1.67 ± 0.47	2.90 ± 1.14
OA score	5.00 ± 4.97	6.67 ± 4.11	13.30 ± 6.51

All data are presented as mean ± SD.

4. Discussion

The ACLT-MMx surgery induced a constant and gradually increasing knee width due to progressive knee joint inflammation. The static hindlimb weight-bearing forces test was conducted to assess pain behavior during OA progression [12-16]. Similar to previous reports [15-16], ACLT-MMx surgery-induced OA resulted in a constant change in weight-bearing asymmetry compared to the normal control (NC) group, which only exhibited acute pain in the initial weeks following the surgical procedure. Additionally, there was no significant difference in body weight (BW) between the NC and OA groups. Throughout the experimental period, rats in the OA group exhibited slowed growth post-ACLT-MMx surgery, but BW steadily increased until the end of the experiment, with no significant difference between groups. Moreover, average daily food and water intake during the experimental period showed no significant difference between the two groups [17-21].

According to our previous study [25-26], an incapacitance tester was used to assess whether the rat's knee joints were experiencing pain or discomfort. It was observed that the pain value significantly increased after ACLT-MMx surgery in the OA group, with higher pressure between the hindlimbs compared to NC group. The width of the rat's knee joint, measured using an electronic digital caliper, was significantly wider in the OA group post-ACLT-MMx surgery, indicating significant knee joint swelling. Pathological examination of the knee joints from each group of rats was performed [22-26]. After 8 weeks post ACLT-MMx surgery, knee joint sections were stained with H&E. H&E staining was used to observe cartilage damage, with histopathological scores indicating greater severity of articular cartilage damage in the OA group compared to the NC group. Overall, the total lesion degree score based on H&E staining was significantly higher in the OA group than that in the NC group, confirming the successful establishment of an ACLT-MMx surgery-induced OA rat model [26]. In this study, after H&E staining, specific areas near the growth plate of the tibia and femurs were selected for the measurement of histomorphometric parameters under a light microscope. The results revealed the grade and stage of OA tibial and femoral articular surface. Regarding the lesion scores of both ends of OA knee joint surfaces, the average OA scores at 1, 2, and 3 month post-operation were 5.17 ± 2.78, 9.33 ± 4.78, and 13.6 ± 6.68, respectively. Taken all results together, the assessment of experimental animals' clinical symptoms and pathological effects of degenerative arthritis were also established. These results further confirm the optimized ACLT-MMx surgery-induced OA rat model.

5. Conclusions

Degenerative arthritis, also known as osteoarthritis (OA), is a prevalent knee joint condition in Taiwan. While mild cases may only result in slight discomfort during walking, severe OA can significantly impair mobility and even lead to loss of basic movement abilities. Unfortunately, once articular cartilage is lost, it cannot be regenerated. Therefore, preventing knee joint degeneration is of paramount importance.

In this study, we employed ACLT-MMx surgery to establish an OA rat model. Through comprehensive data analysis of clinical symptoms and pathological effects of degenerative arthritis, we have successfully created this model. We anticipate that it will serve as a valuable tool for advancing research on OA drugs and the development of therapeutic strategies in the future. By utilizing this model, we aim to gain new insight into the treatment and management of OA, ultimately enhancing the quality of life for individuals affected by this condition.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC 112049 approved by the IACUC ethics committee.

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