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Original Article

Locomotor activity and histological changes observed in a mouse model of knee osteoarthritis

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Abstract. [Purpose] This study aimed to elucidate the changes in locomotor activity in a mouse model of knee osteoarthritis (OA). [Materials and Methods] Fourteen 20-week-old mice were divided into control and OA groups. Knee OA was surgically induced under anesthesia by destabilizing the meniscus. The OA group was reared normally for 8 weeks following surgery, during which OA was induced. Locomotor activity was measured every hour for 8 weeks using an infrared locomotor activity measurement device. Histological changes were evaluated according to the classification-system of Glasson. [Results] Locomotor activity in the OA group significantly decreased up to 2 weeks after surgery. Histological findings in the control group revealed an irregular cartilage surface in a portion of the tibia with no other abnormalities. Contrastingly, those in the OA group had eburnation of the medial femoral condyle, as well as fibrillation and fissures in the medial tibial plateau. Histological scores in the OA group were significantly higher than the control group. [Conclusion] Locomotor activity evaluations, in addition to histological scores and findings, are imperative for studies aiming to clarify the disease state and effect of interventions using mice models.

Key words: Mouse osteoarthritis, Locomotor activity, Histology

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INTRODUCTION

Knee osteoarthritis (KOA) is a multifactorial disease associated with aging, obesity, and mechanical stress. Considering that minute damage generated in the cartilage gradually progresses to degeneration and joint destruction, this disease has been characterized by difficulties in structural improvement. According to the Research on Osteoarthritis Against Disability report, over 30 million Japanese patients aged >40 years suffer from KOA, with its prevalence being expected to increase in the future[1)].

The Japanese Guidelines for the Physical Therapy of KOA[2)] have recommended muscle strengthening and walking for better stabilization of the knee joint. Muscle weakness due to decreased activity increases the amount of stress placed on the knee and decreases activity and quality of life, resulting in a vicious negative cycle. Therefore, maintaining and improving muscle strength and activity are imperative. Treatment guidelines have recommended aerobic exercise, including walking (Grade A), which is supported by Level 1 evidence.

Conversely, considering the mechanism of KOA onset, increasing the activity volume of patients with KOA may lead to excessive mechanical stress, whereas limiting the same may delay the progression of KOA depending on the degree of

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progression. However, only few clinical or basic studies have evaluated the relationship between KOA progression and activity volume. If changes in locomotor activity affect KOA progression, it is necessary to investigate activity volume in addition to histological findings. Therefore, this study aimed to elucidate the changes in locomotor activity in a mouse model of KOA.

MATERIALS AND METHODS

A total of 14 ICR male mice aged 20 weeks (body weight 31.5 ± 10.3 g) were included. All mice were individually housed throughout the experimental period and were allowed free access to water and food in a 12-h light and dark cycle. Animal Care and Use Committee for Kinjo University approved this study (Approval no. 0012).

Following environmental acclimatization for 4 weeks, mice were randomly allocated to an OA group (n=7) and a control group (n=7) reared in a typical manner throughout the experimental period. After placing the mice under anesthesia via inhalation of isoflurane, KOA was surgically induced in the OA group based on previous studies[3, 4]. Accordingly, the articular capsule, medial patellar retinaculum, and vastus medialis were longitudinally incised 1 cm from the inside of the patellar ligament. The patella was laterally inverted, and the knee joint was opened. The medial meniscotibial ligament was then incised to establish a model for destabilization of the meniscus (DMM). Thereafter, the joint was thoroughly cleaned, and the articular capsule, muscle, and skin were sutured. Surgical manipulation was performed on both knees. Following the surgery, normal rearing was performed for 8 weeks in accordance with a previous study, during which KOA was induced in both knee joints.

Locomotor activity was measured every hour for 24 consecutive hours over 8 weeks using an infrared (IR) locomotor activity measurement device (LE8825, PanLab, Barcelona, Spain). This devise contains 32 IR beam sensors (16 each in length and width) in a 45 cm square.

After the experimental period, the mice were euthanized, and the hip joint was transected to collect the hind leg. The collected hind legs underwent tissue fixation for 72 h in 10% neutral buffered formalin solution, followed by decalcification for 72 h using Plank-Rychlo's solution. After decalcification, the knee joint was excised by cutting along the center of the frontal plane. The sample was then neutralized with 5% anhydrous sodium sulfate solution for 72 h, washed in running water, and immersed in 100% alcohol for 3 h for degreasing. Paraffin embedding was performed using paraffin blocks that were sliced thinly (approximately 3 μm) with a microtome (TU-213, Yamato, Saitama, Japan). Samples were then stained with safranin-O fast green to create tissue specimens, which were observed under an optical microscope (BX53, Olympus, Tokyo, Japan). The right knee was used for histological findings and left knee was used for histological scoring. Histological changes in the knees were classified using the semi-quantitative scoring system of Glasson et al.[5] wherein a score of 0 represents normal cartilage; 0.5, loss of safranin-O without structural changes; 1, small fibrillations without loss of cartilage; 2, vertical clefts down to the layer immediately below the superficial layer and some loss of surface lamina; 3, vertical clefts/erosion to the calcified cartilage extending <25% of the articular surface; 4, vertical clefts/erosion to the calcified cartilage extending 25–50% of the articular surface; 5, vertical clefts/erosion to the calcified cartilage extending 50–75% of the articular surface; and 6, vertical clefts/erosion to the calcified cartilage extending >75% of the articular surface. The scores were evaluated by blinded person who was not involved in this study.

The changes of locomotor activity between both groups was statistically tested by two-way repeated measure analysis of variance after Shapiro-Wilk test and F-test using statistical software R (version 3.3.2). The main effect was then analyzed as a post-hoc test using Welch's t-test with Bonferroni correction. And histological scores were compared using Mann-Whitney U test. The level of significance was set as 0.05.

RESULTS

The locomotor activity value of the mice before surgery (at the age of 24 weeks) was 19,449 ± 373 times. In the OA group, the value decreased significantly only up to 2 weeks after surgery ($p<0.01$). No differences between both groups had been observed from postoperative 3 week until the end of the study period (Table 1).

Histological findings showed an articular cartilage layer stained red with safranin in the control group. The cartilage surface appeared irregular in a portion of the tibia with no other abnormalities. On the other hand, the OA group showed safranin hypochromia and deviations in the medial meniscus, with extensive eburnation of the medial femoral condyle and fibrosis and fissures in the medial tibial plateau (Fig. 1), which indicated KOA pathology.

Histological scores were 0.5 (0–0.5) and 4 (2–6) for the femoral cartilage and 0 (0–0.5) and 3 (1–4) for the tibial cartilage in the control and OA groups, respectively (Table 2). In all measurements, the OA group showed significantly higher scores than the control group ($p<0.05$).

DISCUSSION

KOA progression is caused by a breakdown in articular cartilage homeostasis due to excessive mechanical stress. Accordingly, cartilage damage gradually progresses to cartilage degeneration and joint destruction. Histological findings have revealed the loss of extracellular cartilage matrix, presence of superficial fibrosis, thinning and cracking of the articular

Table 1. Locomotor activity

	Contol	OA	p value
Before surgery	19,449 ± 373		
1 week after surgery	20,555 ± 269	9,040 ± 551	<0.00001**
2 weeks	19,054 ± 392	10,062 ± 704	<0.00001**
3 weeks	19,958 ± 883	17,999 ± 945	0.053
4 weeks	18,338 ± 881	18,344 ± 861	0.315
5 weeks	19,006 ± 449	18,044 ± 758	0.070
6 weeks	18,291 ± 392	18,924 ± 840	0.257
7 weeks	18,894 ± 288	18,845 ± 632	0.746
8 weeks	18,991 ± 402	18,777 ± 750	0.858

**Significant difference from control (p<0.01).

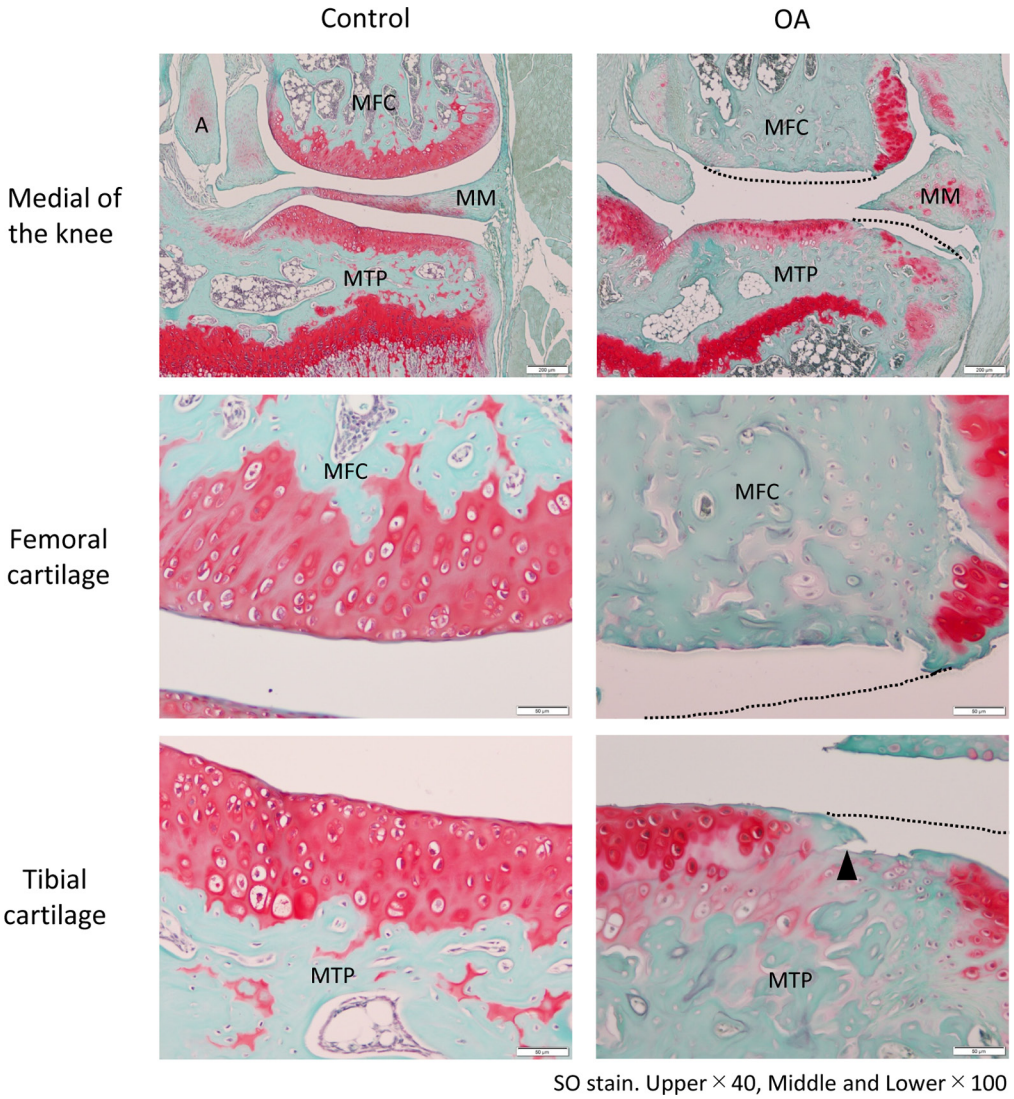

Fig. 1. Histological findings.
Upper: medial knee (femur: above; tibia: below); middle: magnified image of the femoral cartilage; lower: magnified image of the tibial cartilage. Histological findings included prolapsed medial meniscus, irregular surface layer of the cartilage, fibrillation (▲) and eburnation (broken line is lost cartilage) in the osteoarthritis group. Decreased cartilage staining (femur and tibia) was also observed (A: anterior cruciate ligament, MM: medial meniscus, MFC: medial femoral condyle, MTP: medial tibial plateau).

Table 2. Histological scores

Femoral	Number of sumple							
	1	2	3	4	5	6	7	p value
Control	0	0	0	0.5	0.5	0.5	0.5	
OA	2	4	4	4	4	5	6	0.0017**

Tibial	Number of sumple							
	1	2	3	4	5	6	7	p value
Control	0	0	0	0	0.5	0.5	0.5	
OA	1	2	3	4	4	3	4	0.0477*

**Significant difference from control ($p<0.01$).
*Significant difference from control ($p<0.05$).
Data are shown as the score of scoring system of Glasson (0, represents normal cartilage; 0.5, loss of safranin-O without structural changes; 1, small fibrillations without loss of cartilage; 2, vertical clefts down to the layer immediately below the superficial layer and some loss of surface lamina; 3, vertical clefts/erosion to the calcified cartilage extending <25% of the articular surface; 4, vertical clefts/erosion to the calcified cartilage extending 25–50% of the articular surface; 5, vertical clefts/erosion to the calcified cartilage extending 50–75% of the articular surface; and 6, vertical clefts/erosion to the calcified cartilage extending >75% of the articular surface).

cartilage layer, chondrocyte clustering and cell death, and osteophyte formation at the periphery of the joint[5, 6]. Previous studies have reported that such findings occurred 8 weeks after surgery, which was consistent with that observed herein.

In hopes of studying the responses of OA cartilage to mechanical stress, Iijima et al. investigated the prevention of subchondral bone degeneration by treadmill walking (12 m/min, 30 min/day, 5/week) using a rat model of KOA[7]. They ultimately concluded that treadmill walking increased bone morphogenetic proteins and can prevent OA-induced subchondral bone lesions. By contrast, Hashimoto et al. reported that administering treadmill walking interventions involving running for at least 7.5 min to intact mice resulted in KOA from 2 weeks onward[8]. The aforementioned data therefore suggest that degeneration is caused by the generation of a load that exceeds the maximum capacity of the cartilage.

Kim et al. evaluated KOA progression and locomotor activity in a mouse DMM model wherein animals were placed in normal (260L × 200W × 130H) or restricted (75L × 40W × 200H) cages[9]. The authors found that animals in restricted cages without any treadmill intervention had the least severe histological scores and findings, whereas those in normal cages had the most severe. Accordingly, limiting locomotor activity has been suggested to delay KOA progression, whereas increasing locomotor activity has been suggested to increase it.

Histologic findings in this study were similar to previous studies[3–5]. Interestingly, the present study showed that OA seemed to have progressed despite the comparable locomotor activity between the control and OA groups, indicating that maintaining a certain activity volume appears to lead to excessive mechanical stress on the knee joint, thereby gradually causing KOA. Previous studies[8, 9] have shown that OA lesions were minimal when locomotor activity was reduced in a restricted cage, suggesting that locomotor activity is involved in the progression of OA. Notably, the previous studies using a KOA model displayed variations in the observed histological scores and findings[3, 4], which appeared to be have been caused by differences in the locomotor activity administered to the animals. Therefore, locomotor activity evaluations in addition to histological scores and findings are imperative for studies aiming to clarify the disease state and effect of interventions using a DMM model of KOA. Finally, a simple comparison was difficult because no group was kept in a restricted cage after surgery in this study. The relationship between the changes in locomotor activity and the progress of KOA needs to be considered in more detail in the future.

Presentation at a conference

The part of our research was presented in the WCPT Congress 2019 in Geneva.

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Conflict of interest

There are no conflict of interest relating to this study.

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