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Joint unloading inhibits articular cartilage degeneration in knee joints of a monosodium iodoacetate-induced rat model of osteoarthritis

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SUMMARY

Objective: The aim of the study was to examine how mechanical unloading affects articular cartilage degeneration in the patellofemoral (PF) and tibiofemoral (TF) joints of a monosodium iodoacetate (MIA)-induced rat model of osteoarthritis (OA).
Design: The study involved 60 male rats. OA was induced by intra-articular injecting MIA into both knee joints. All animals were equally divided into two groups: sedentary (SE) and hindlimb unloading (HU) groups. Histopathological changes in the articular cartilage of the PF and TF joints were evaluated using the Osteoarthritis Research Society International (OARSI) score and modified Mankin score at 2 and 4 weeks after MIA injection.
Results: In the SE and HU groups, representative histopathological changes in OA were detected in the PF and TF joints. The OARSI and modified Mankin scores for the PF and TF joints tended to increase over time after the injection of 0.2 mg or 1.0 mg of MIA in the SE and HU groups. Both the scores for the HU group were significantly lower than those for the SE group [OARSI score: $P < 0.0001$ (1.0-mg injection at 4 weeks); modified Mankin score: $P = 0.0116$ (0.2-mg injection at 4 weeks); $P = 0.0004$ and < 0.0001 (1.0-mg injection at 2 and 4 weeks, respectively)].
Conclusion: This study revealed new histological evidence that indicates that unloading condition suppresses articular cartilage degeneration and is beneficial in many areas of basal and clinical research involving OA.

Introduction

Osteoarthritis (OA) of the knee is a major cause of pain and locomotor disability worldwide[1]. Numerous factors contribute to OA, including aging, obesity, genetics, and mechanical loading[2,3]. Excessive mechanical stress is also an important factor in OA onset[4,5]. Various guidelines and treatment methods for OA have been developed based on these facts, including the guidelines published in 2014 by the Osteoarthritis Research Society International (OARSI) on the non-surgical treatment of OA[1]. This guideline includes weight management (i.e., weight loss) as the core treatment for all individuals[1]. The treatment guidelines published in 2014 by the National Institute for Health and Care Excellence of the United Kingdom National Health Service and those published in 2013 by the American Academy of Orthopedic Surgeons also highlight the importance of weight loss for treating OA[6,7]. High tibial osteotomy during orthopedic surgery, exercise therapy, gait correction, walking aids, and foot and knee orthoses in physical therapy can help attenuate the mechanical stress on knee joints[1,8].

Mechanical stress plays a significant role in cartilage metabolism[4]. Many researchers examined the influence of loading on cartilage metabolism and revealed that appropriate mechanical stress levels stimulate cartilage metabolism[4]. For example, applying appropriate mechanical stress to the articular cartilage stimulates transforming growth factor-1 and sex-determining region Y-box 9 expressions and increases type II collagen and aggrecan production[9,10]. Moderate exercise inhibits OA

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progression[11–13]. Conversely, excessive mechanical stress causes the degeneration and destruction of normal cartilage and accelerates OA progression by inducing morphological, molecular, and mechanical changes in cells and the matrix, leading to softening, fibrillation, ulceration, and/or loss of cartilage[5,11,12,14]. Furthermore, reduced joint loading or motion is associated with atrophy and degeneration of normal cartilage, including cartilage thinning, tissue softening, and proteoglycan content reduction[15,16].

The monosodium iodoacetate (MIA) model is an established and a commonly used model of OA[17]. Injecting the metabolic inhibitor of MIA into the joints inhibits glyceraldehye-3-phosphate dehydrogenase activity in chondrocytes, resulting in the disruption of glycolysis and eventual cell death[18]. Although this model has no correlation with the pathogenesis of any type of human OA, the histological changes in the articular cartilage induced by MIA resemble those induced by human OA[18]. The MIA model is a minimally invasive, quick, and easy to perform and provides reproducible results; moreover, it can be used in animals exhibiting signs of OA-related pain[18]. In addition, the onset, progression, and severity of OA can be easily controlled in this model by changing MIA dose, rendering it useful to study disease progression[19].

Hindlimb unloading (HU) is a well-established method to simulate the effects of microgravity or prolonged rest on the skeletal system[20]. The Morey-Holton method for HU is a widely accepted National Aeronautics and Space Administration ground-based model for studying disuse atrophy in rodents[21,22]. Ferreira *et al.* further modified this method for HU and reported that it was a useful, simple alternative to the traditional Morey-Holton HU technique[22]. They also reported that the utility of the modified method was evident with a maintenance in animal body weight, comparable adrenal gland weights, and soleus atrophy following HU[22]. Therefore, recently, the tail suspension method is being applied for studying the articular cartilage[15].

Although various OA treatment guidelines recommend that the loads placed on patients' joints should be reduced, our literature search failed to reveal any studies that analyzed whether OA progression is suppressed by reducing the loads placed on joints or the histological impact of such an approach. The purpose of this study was to examine how mechanical unloading affects articular cartilage degeneration in the patellofemoral (PF) and tibiofemoral (TF) joints of a MIA-induced rat model of OA.

Methods

Experimental animals and animal care

This study was approved by the animal research committee of Kanazawa University Graduate School of Medicine in Kanazawa, Japan (approval No.: 143259, 173831 and 183933) and was conducted in accordance with the ARRIVE guidelines[23] and the guidelines for the care and use of laboratory animals at Kanazawa University.

Sixty male Wistar rats (9-week-old) were used for this study. The animals were housed in normal conditions for 1 week before the start of the experiments for acclimatization. One or two rats were housed per cage in a sanitary ventilated room under controlled temperature and humidity conditions and a 12-h/12-h light–dark cycle. Food and water were provided *ad libitum*.

The rats were equally divided into two groups: the sedentary (SE) and HU groups ($n = 30$ per group). In the SE group, the rats were kept under normal physiological conditions and were allowed to walk freely in standard cages. In the HU group, the rats were subjected to tail suspension-based HU throughout each experiment. Rats in the HU group were allowed to walk freely using only their forelimbs; the details of the tail suspension method are described below. The 30 animals in each group were assigned to three subgroups depending on the dose of MIA administered; no administration, 0.2, and 1.0 mg ($n = 10$ per subgroup). Therefore, the six subgroups were as follows: SE, HU, SE + MIA0.2 mg, HU + MIA0.2 mg, SE + MIA1.0 mg, and HU + MIA1.0 mg. Furthermore, 10 rats in each subgroup were later randomly allocated to one of the two groups: 2 and 4 weeks after MIA injection ($n = 5$ each). After the injection of MIA, no further interventions, such as range of motion exercise or treadmill running, were performed on any animal during the experimental period. No analgesics or anti-inflammatory drugs were used.

This was a study concomitant to our previous study[24]. A portion of the data was derived from our previous study. Specifically, the previous study reported data regarding body weight and OARSI scores in the PF and TF joints of the walking group at 2 and 4 weeks after injecting 0.2 and 1.0 mg MIA. However, the present study reported data regarding body weight and OARSI scores in the PF and TF joints after injecting 0.2 and 1.0 mg SE + MIA at 2 and 4 weeks.

Creation of OA model

OA was induced by intra-articular injection of MIA. MIA was dissolved in 30 μL of sterile saline, and the MIA dose was set at 0.2 or 1.0 mg. MIA was injected into both the knees. For details see Supplementary Methods.

Hindlimb suspension

The HU group was subjected to tail suspension-based HU throughout the experiment. In the present study, hindlimb suspension was performed according to Andries Ferreira's modified tail suspension method[15,22,25]. Briefly, under inhalation-induced anesthesia with isoflurane, the rat's tail was disinfected. A sterile steel wire was then used to drill into the proximal part of the coccyx, in which the wire remained, and the steel wire was shaped into a ring so that it could subsequently be used for the suspension procedure. The tail ring was then connected to a track hung from the ceiling of the cage using a string, enabling the animals to roam freely on their forelimbs throughout the cage. The head-down tilt angle was monitored throughout the experimental period and remained at approximately 30°[15].

Histological preparation

After the experimental period, the animals were euthanized via intraperitoneal injection of a lethal dose of sodium pentobarbital, and both hindlimbs were disarticulated at the hip joint. The right and left knee joints were sagittally and frontally excised to evaluate the articular cartilage in the PF and TF joints, respectively. Decalcified paraffin-embedded specimens of the sagittal and frontal planes were sliced serially at 3 μm and were separately stained with hematoxylin and eosin and 0.1% safranin O fast green. For details see Supplementary Methods.

Selection of the regions for assessment

To evaluate the degree of OA in the PF joint, the regions of the articular cartilage associated with the femoral patella groove and patella were selected [Fig. 1-(A)]. To determine the degree of OA in the TF joint, the central area of the articular cartilage of the tibia and femur at the medial TF joint were selected for assessment [Fig. 1-(B)]. For details see Supplementary Method.

Histological analysis

To clarify the histopathological changes that occur in OA, we quantitatively evaluated these changes using the OA cartilage

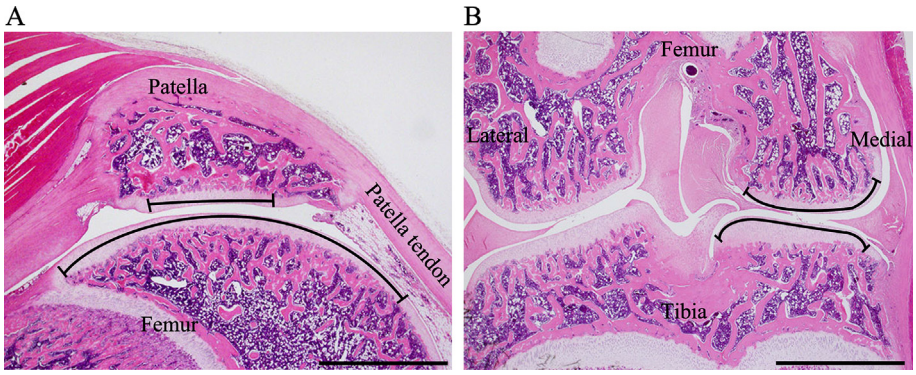

Fig. 1. The regions of the PF and TF joints selected for histopathological assessments. A: The patellar and femoral articular cartilages were evaluated on a sagittal section from the center of the PF joint. B: The tibial and femoral articular cartilages were assessed on a frontal section from the center of the TF joint. Scale bar = 2 mm.

histopathology assessment system (OARSI score)[24,26] and modified Mankin score[27–29]. The OARSI score was established by Pritzker *et al.*; the total score ranges from 0 to 24, with higher values indicating more advanced cartilage degeneration[26]. The Mankin score is a histological scoring system for the quality of the articular cartilage in OA[27,28]. We modified this scoring system according to a previous study (Table I)[29]. The modified Mankin score comprises structure, cellularity, safranin O staining intensity, and tidemark integrity; the total score was 0–15, with higher values indicating more severe cartilage degeneration.

Each of the above four regions of the articular cartilage was evaluated for both knees (the patella and femur in the PF joint of the right knee and tibia and femur in the TF joint of the left knee). One section containing the abovementioned specified region in the PF or TF joint [Fig. 1-(A) and (B)] was chosen. The histological features and scores using the two scoring systems were evaluated and determined by two blinded and trained independent observers (M.H., a pathologist and I.T.). Interclass correlation coefficients for the intra- and inter-rater reliability with 95% confidence intervals were excellent; OARSI score: 0.94 (0.92–0.95) and 0.91 (0.89–0.93), modified Mankin score: 0.98 (0.97–0.98) and 0.97 (0.96–0.98), respectively.

Table I
The modified Mankin grading system

Structure	
Normal	0
Surface irregularities	1
Pannus and surface irregularities	2
Clefts to transitional zone	3
Clefts to radial zone	4
Clefts to calcified zone	5
Complete disorganization	6
Cellularity	
Normal	0
Increase or slight decrease	1
Moderate decrease	2
Severe decrease	3
No cells	4
Safranin O staining	
Normal	0
Slight decrease	1
Moderate decrease	2
Severe decrease	3
No staining	4
Tidemark integrity	
Normal	0
Disappearance or invasion by vessels	1

Statistical analysis

All statistical analyses were performed using the JMP 14 software (SAS Institute, Cary, NC, USA). All data were statistically analyzed as parametric data. The sample size was 5 for each group; for the OARSI score and modified Mankin score, each of 5 animals contributed with four measurements. In addition, descriptive statistics were calculated as the median with interquartile range for the OARSI and modified Mankin scores and as the mean with standard deviation for body weight. We indicated graphical results for the two scores using two colors and four types of shapes as follows: black and white, the SE and HU group; the circle, triangle, square, and rhombus, the score of the patella and femur in the PF joint and the tibia and femur in the TF joint, respectively. We considered $P < 0.05$ as statistically significant for all analyses; exact P values are shown in the figures. Regarding the body weight, we performed the analysis of variance (ANOVA), followed by the post-hoc Tukey's honestly significant difference test for all groups. Regarding the OARSI and modified Mankin scores, the differences among the groups, considering the within- and between-group variance, were evaluated using ANOVA for repeated measurements, and after residuals diagnosis, post-hoc Tukey's honestly significant difference test was subsequently used.

Regarding the OARSI and modified Mankin scores, details of the statistical analysis method are as follows: four scores were obtained from 1 rat in each scoring system—these scores corresponded to the patella and femur in the PF joint and the tibia and femur in the TF joint. In addition, to elucidate the site-specific impact of unloading on articular cartilage degeneration, we conducted four-way ANOVA for repeated measurements (factors: loading, MIA dose, experimental period, and site). Consequently, the primary effect of the site was not detected (site, $P = 0.1143$; others, $P < 0.0001$ for the OARSI score and $P = 0.4272$; others $P < 0.0005$ for the Mankin score); these findings implied that it was statistically impossible to analyze the site-specific impact and that no difference was observed in the score depending on the site. Hence, the data of the four sites obtained from 1 rat were considered to correspond to histological changes that occurred in the entire knee joint, not at a specific site.

Next, we performed three-way ANOVA for repeated measurements (factors: loading, MIA dose, and experimental period), which confirmed that the primary effect was observed for all three factors (all $P < 0.0001$ for both OARSI and Mankin scores) and that the interaction effect (loading-MIA dose, loading-experimental period, MIA dose-experimental period, loading-MIA dose-experimental period) was detected for all four factor interactions (all $P < 0.0001$ for the OARSI score and all $P < 0.025$ for the Mankin score).

Finally, we conducted the post-hoc Tukey's honestly significant difference test for all 12 groups (all two conditions of loading, all three of MIA dose, and all two of the experimental period).

Results

Within the first few minutes after MIA injection, all the animals regained consciousness and started to move. None of the rats exhibited any signs of knee infection or swelling, and none died during the experimental period. Inflammation was macroscopically and microscopically well-controlled. In the HU group, none of the rats were dropped by the tail suspension apparatus during the experimental period. The body weights of the rats throughout the experimental period are shown in Supplementary Result 1. In all the groups and subgroups, increases in the body weight were observed over time.

Histopathological changes in the PF joint

Among the rats injected with 0.2 mg MIA, the surfaces of the patellar and femoral articular cartilage were smooth at 2 and 4 weeks in both the SE and HU groups [Fig. 2-(A)]. In both the groups, weak safranin O staining of the cartilage matrix was detected in all regions of the patella and femur at 2 weeks. At 4 weeks, staining of the cartilage matrix demonstrated slight regeneration at the margins of the articular cartilage and in the contact area between the patellar and femoral articular cartilage.

Among the rats in the SE group injected with 1.0 mg MIA, fibrillation was observed in the femur; however, no articular cartilage surface irregularities were detected in the patella at 2 weeks [Fig. 2-(B)]. At 4 weeks, fibrillation, fissuring, and eburnation were observed in the patella and femur. In the HU group, no histological changes other than fibrillation were observed at 2 or 4 weeks. In all the regions of the patella and femur, safranin O staining resulted in no or weak staining of the cartilage matrix at 2 and 4 weeks in both the groups.

Figure 3-(A) shows representative histopathological features of the articular cartilage in the PF joint in the SE and HU groups at 4 weeks after the injection of 1.0 mg MIA. A summary of the histological findings of OA in the PF joint is shown in Supplementary Result 2.

Histopathological changes in the TF joint

In the 0.2 mg MIA subgroup of the SE group, fibrillation, fissuring, and eburnation were observed at 4 weeks [Fig. 4-(A)]. In the HU group, the surfaces of the tibial and femoral articular cartilage were smooth at 2 and 4 weeks. In both the groups, weak safranin O matrix staining was detected in all regions at 2 weeks. In the SE group, matrix staining showed slight regeneration at the margins of the tibia and femur at 4 weeks. In the HU group, reduced staining of the matrix was observed at 4 weeks.

In the 1.0 mg MIA subgroup of the SE group, the tibia and femur displayed fibrillation at 2 weeks and fissuring, eburnation, and denudation at 4 weeks [Fig. 4-(B)]. In the HU group, the surface of

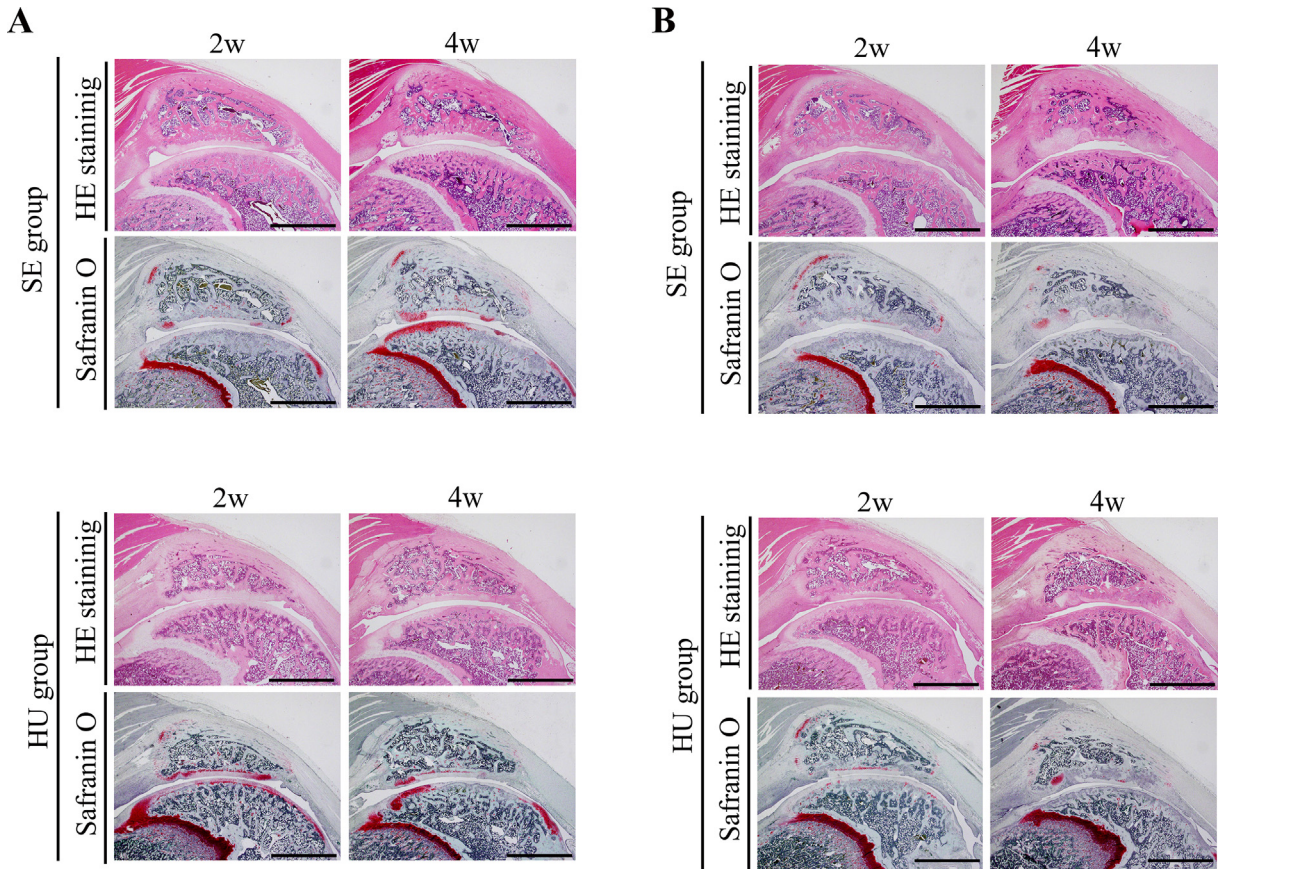

Fig. 2. The histopathological overview of the PF joint of the SE and hindlimb unloading (HU) groups. A: Histopathological changes of the articular cartilage after the injection of 0.2 mg monosodium iodoacetate (MIA). No whole-joint deformation was observed during the study period. Partial safranin O staining of the cartilage matrix was observed in the patella and femur in both the groups during the study period. The surface of the articular cartilage was smooth at 2 and 4 weeks in both the groups.B: Histopathological changes of the articular cartilage after the injection of 1.0 mg MIA. No whole-joint deformation was observed during the study period. In both groups, no or weak safranin O staining of the cartilage matrix was noted in the patella and femur throughout the study period. In only the SE group, osteoarthritic histological changes, such as fibrillation, fissuring, and erosion, were seen in the patella and femur after 4 weeks. Scale bar = 2 mm.

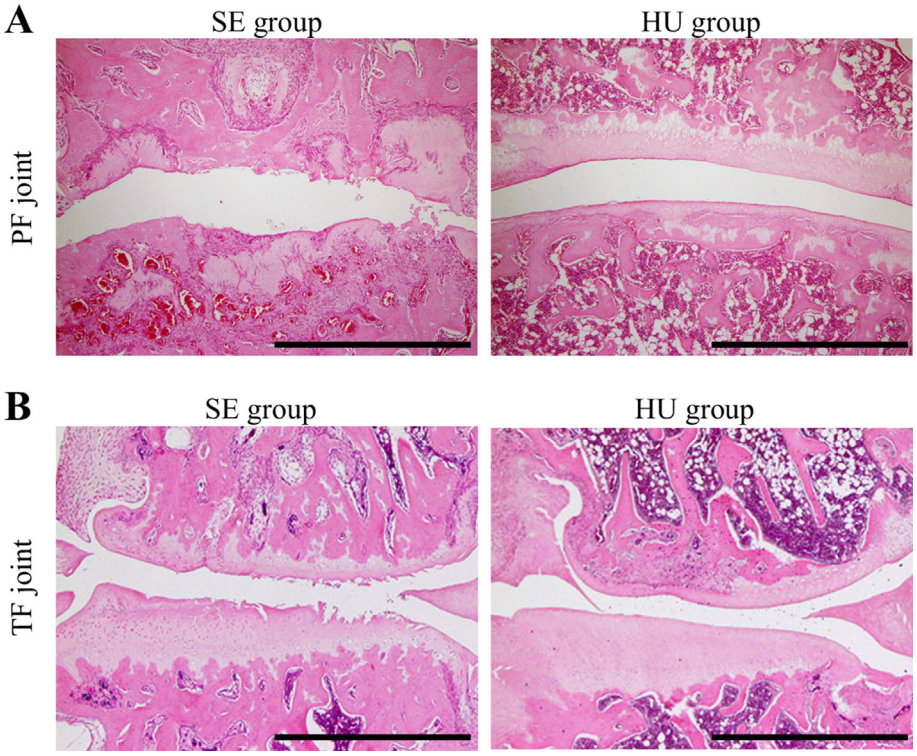

Fig. 3. Highly-magnified representative histopathological features of the articular cartilage in the PF and TF joints. A: Histopathological images of the PF joint at 4 weeks after the injection of 1.0 mg MIA.In the SE group, erosion and denudation of the PF joint were observed. In the HU group, the surface of the articular cartilage was smooth without fibrillation or fissuring. B: Histopathological images in the TF joint at 4 weeks after injecting 1.0 mg MIA. In the SE group, fibrillation, fissuring, and erosion in the TF joint were observed. In the HU group, the surface of the articular cartilage was smooth, and no fibrillation or fissuring was detected. Scale bar = 1 mm.

the articular cartilage remained smooth at 2 and 4 weeks. In both the groups, weak safranin O staining of the matrix was detected in all the regions at 2 and 4 weeks.

Figure 3-(B) shows representative histopathological features of the articular cartilage in the TF joint in the SE and HU groups at 4 weeks after the injection of 1.0 mg MIA. A summary of the histological findings of OA in the TF joint is shown in Supplementary Result 3.

OARSI score

A comparison of the OARSI scores between the SE and HU groups is shown in Fig. 5 and Supplementary Result 4 and 6. The score for all MIA doses and experimental periods in the HU group remained low. However, in the SE group, the score increased by MIA dose and time dependency. There was a significant difference between the OARSI scores of the SE and HU groups of the 1.0 mg MIA injection at 4 weeks.

Modified Mankin score

A comparison of the modified Mankin scores between the SE and HU groups is shown in Fig. 6 and Supplementary Result 5 and 6. In no MIA administration subgroup, the score in the HU group at 2 and 4 weeks was higher than that in the SE group. In 0.2 and 1.0 mg MIA administration, the score of the SE and HU groups increased with MIA dose and time dependency. The score in the HU group was significantly lower than that in the SE group of 0.2 mg MIA injection at 4 weeks and 1.0 mg MIA injection at 2 and 4 weeks.

Discussion

Before discussing the results of the present study, several aspects need to be discussed. First, the MIA-induced rat model of OA is well-established and rapidly induces progressive OA[18]. For example, Guingamp *et al.* reported that injection of 0.3 mg MIA resulted in complete articular cartilage erosion and osteophyte formation within 30 days[30]. In surgically induced OA models, including those involving anterior cruciate ligament transection or medial meniscus destabilization, the induced condition did not progress to end-stage OA within 4 weeks[11,12,31,32]. In the present study, a hindlimb suspension-based intervention was employed and the experimental period was limited because we supposed that it would be difficult to follow the rats for long periods. Therefore, we selected the MIA-induced OA model, which is a minimally invasive, quick, and easy and provides reproducible results; moreover, it is useful to study disease progression[18,19].

Second, many studies involving hindlimb suspension have been reported. There are several hindlimb suspension methods, including the Morey-Holton method[20–22,33,34] and a method involving a trunk jacket[35]. In the present study, we adopted Ferreira's modified tail suspension method, in which exterior tail fixation is performed using a Kirschner wire[22]. This method was described in our previous study[25]. It is associated with a low risk of infection and does not cause marked weight loss. Furthermore, because the rats are suspended using a Kirschner wire, the risk of their being dropped during the suspension period is low, and it is possible to suspend the animals for long periods. In the present study, using this method for unloading the hindlimb, the weight of the rats increased; no rats were dropped during the experimental period.

Third, although it is obvious that mechanical stress induced by loading decreases with hindlimb suspension, the influence of unloading on the articular cartilage has not yet been sufficiently elucidated. Previous studies reporting on the influence of joint unloading without joint-motion restriction on the articular cartilage exhibit no consensus regarding histopathological changes in

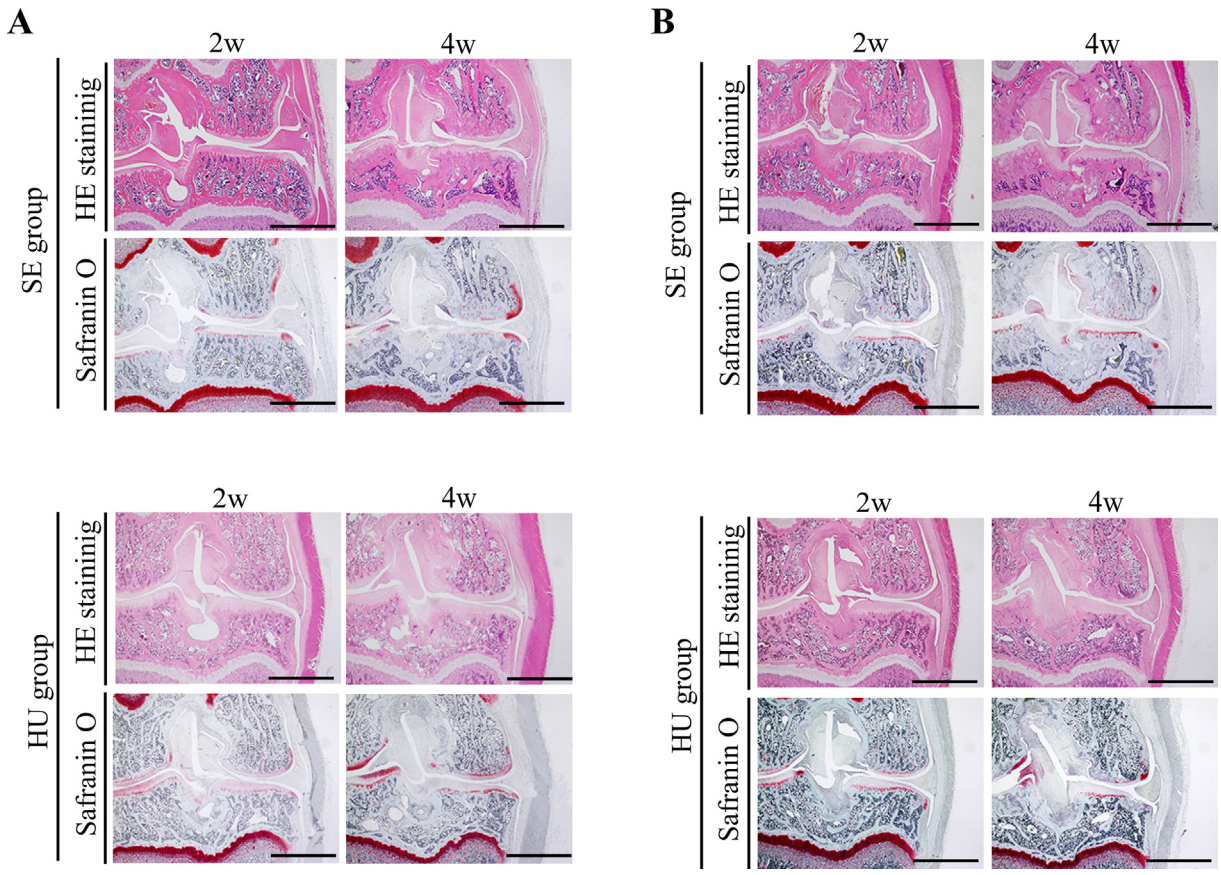

Fig. 4. Histopathological overview of the tibiofemoral (TF) joint of the SE and HU groups. A: Histopathological changes of the articular cartilage after the injection of 0.2 mg MIA.No whole-joint deformation was observed during the study period. In the SE group, safranin O staining of the matrix revealed slight regeneration at the margins of the tibia and femur at 4 weeks. No histological changes other than fibrillation were observed in the femur in either group during the study period. In the SE group, fissuring and erosion were detected in the tibia at 4 weeks. In the HU group, no histological changes other than fibrillation were noted. B: Histopathological changes of the articular cartilage after the injection of 1.0 mg MIA. No whole-joint deformation was observed during the study period. In the SE group, fibrillation and erosion were observed at 4 weeks. In the HU group, the surface of the articular cartilage was smooth at 2 and 4 weeks. Scale bar = 2 mm.

the articular cartilage (the proteoglycan content, number of chondrocytes, thickness of cartilage, chondrocyte clusters, and tidemark and subchondral vessels)[15]. Therefore, in the present study, the number of chondrocytes and matrix stainability by safranin O decreased in the HU group with no MIA administration; as a result, the modified Mankin score in this group significantly increased at 2 and 4 weeks. From this result, we presumed that unloading may have some negative effect on the normal articular cartilage.

Fourth, in the present study, after conducting the primary experiment, additional experiments were conducted. Similar staining conditions and environmental factors (laboratory, dyeing solution, room temperature, and used goods) were used to avoid any obvious differences in staining between the two experiments. Moreover, before conducting the experiments, we confirmed the absence of apparent differences in the staining intensity. Although there was a possibility that a very minor difference in staining intensity occurred, as there was no item directly measuring the staining intensity in the histological analysis, there was no obvious problem affecting the results of this study. In histological evaluation, the evaluators were blinded to all the experimental conditions, including group assignment, MIA dose, and experimental period; moreover, the intra- and inter-rater reliability was very high. Therefore, it was impossible to simultaneously perform histological analysis and evaluation; however, with ingenuity and consideration, it was possible to perform highly scientific experiment with the assurance of objectivity and reproducibility.

Based on the abovementioned backgrounds, we examined how mechanical unloading affects articular cartilage degeneration in the PF and TF joints. We observed characteristic histopathological changes of OA in the PF and TF joints in the SE and HU groups, including fibrillation, fissuring, and erosion, as in the results described in previous study[19,23,30,36–38]. Furthermore, the degree of histological changes in the HU group was significantly smaller than that in the SE group in terms of the OARSI and modified Mankin scores. Regarding the metabolism of the articular cartilage, the balance and relationship between unloading, loading, and exercise have been still unclear, and some animal studies have demonstrated the influence of mechanical stress on OA progression using MIA injection, including treadmill running. However, the results were inconclusive[39,40]. To the best of our knowledge, the present study is the first to assess the influence of joint unloading on articular cartilage degeneration using rat model of OA in the PF and TF joints. Therefore, our data provide new histopathological evidence regarding the pathogenesis of OA and support the current clinical guidelines for its treatment. However, long-term unloading may have negative effects on the articular cartilage; thus, further studies regarding HU are required to evaluate appropriate loading quantity and to clarify the influence of mechanical stress on the metabolism of the articular cartilage.

The present study had two potential limitations. Firstly, the tail suspension method achieved unloading on the knee joint owing to the body weight such as during walking or maintaining posture

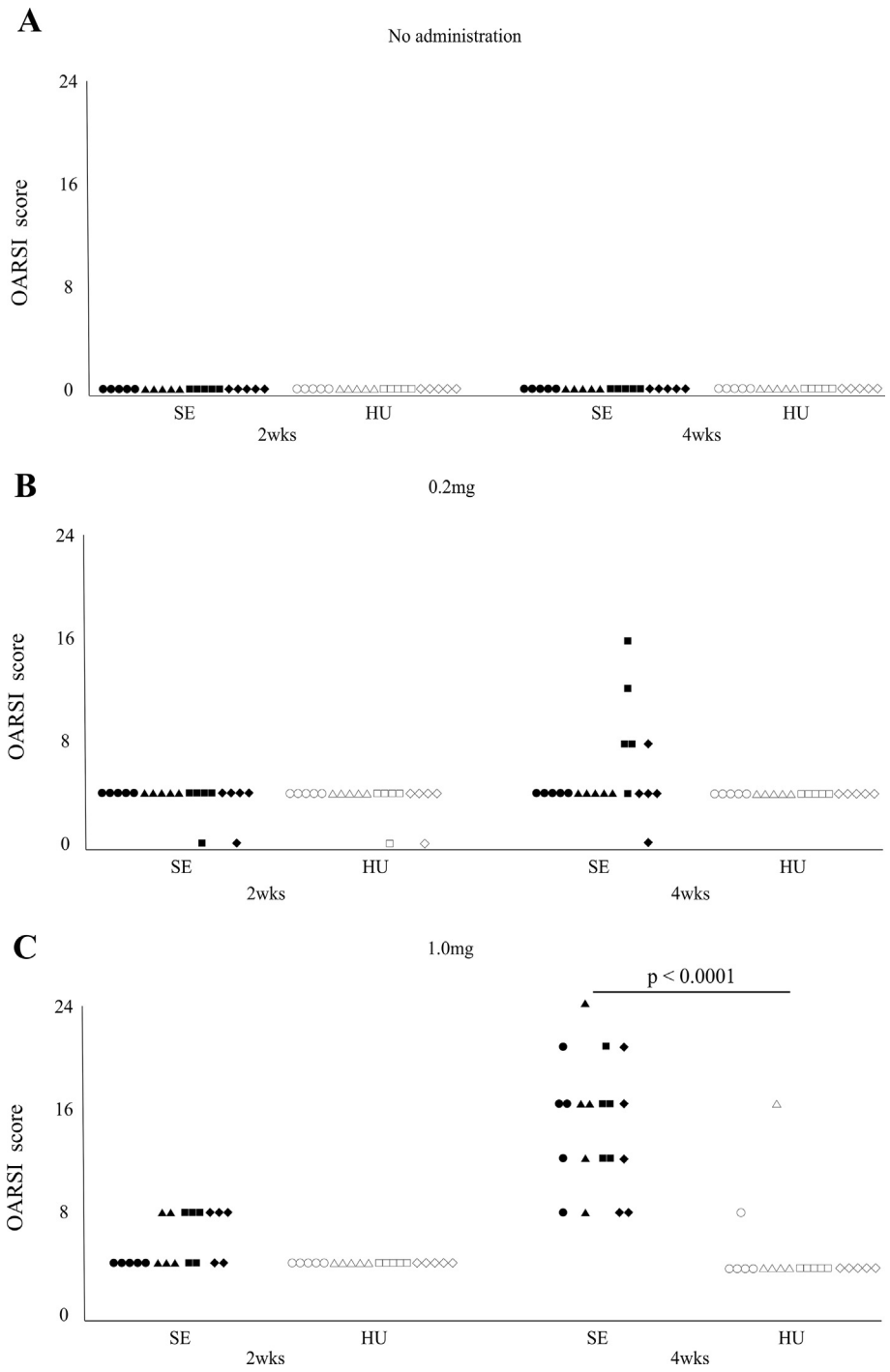

Fig. 5. Time-dependent changes in the Osteoarthritis Research Society International (OARSI) score of no (A), 0.2 mg (B) and 1.0 mg (C) MIA administration. There were no statistically significant intergroup differences in the OARSI scores at 2 and 4 weeks of no and 0.2 mg MIA administration. After injecting 1.0 mg MIA, a significant difference was noted in the OARSI scores at 4 weeks ($P < 0.0001$). Each shape indicates each specimen score; black and white shapes indicate the SE and HU groups, respectively. The circle, triangle, square and rhombus mean the score of the patella and femur in the PF joint and tibia and femur in the TF joint, respectively.

during the experimental period. However, the joint motion (with or without muscle contraction) is not restricted and mechanical stress, such as shear and compressive forces, on the articular cartilage cannot be completely eliminated. These mechanical stresses affect the metabolism of the normal articular cartilage[41,42]; moreover, Kobayashi *et al.* reported that the PF and TF joints exhibit various structural, pathomechanical, and clinical characteristics[43]. Therefore, it may be possible that the environmental conditions of this experiment, such as the unloading condition where the joint motion (with or without muscle contraction) is possible, may have influenced the difference of suppression effect exhibited by joints and sites.

Secondly, we used a small sample size for statistically analyzing site-specific difference. As mentioned above, PF and TF joints exhibit structural and kinematic differences[43]. Clark suggested three possible factors that might affect the progression of OA: (1) loading duration; (2) histological, material, and compositional properties; and (3) anabolic or catabolic metabolism within the

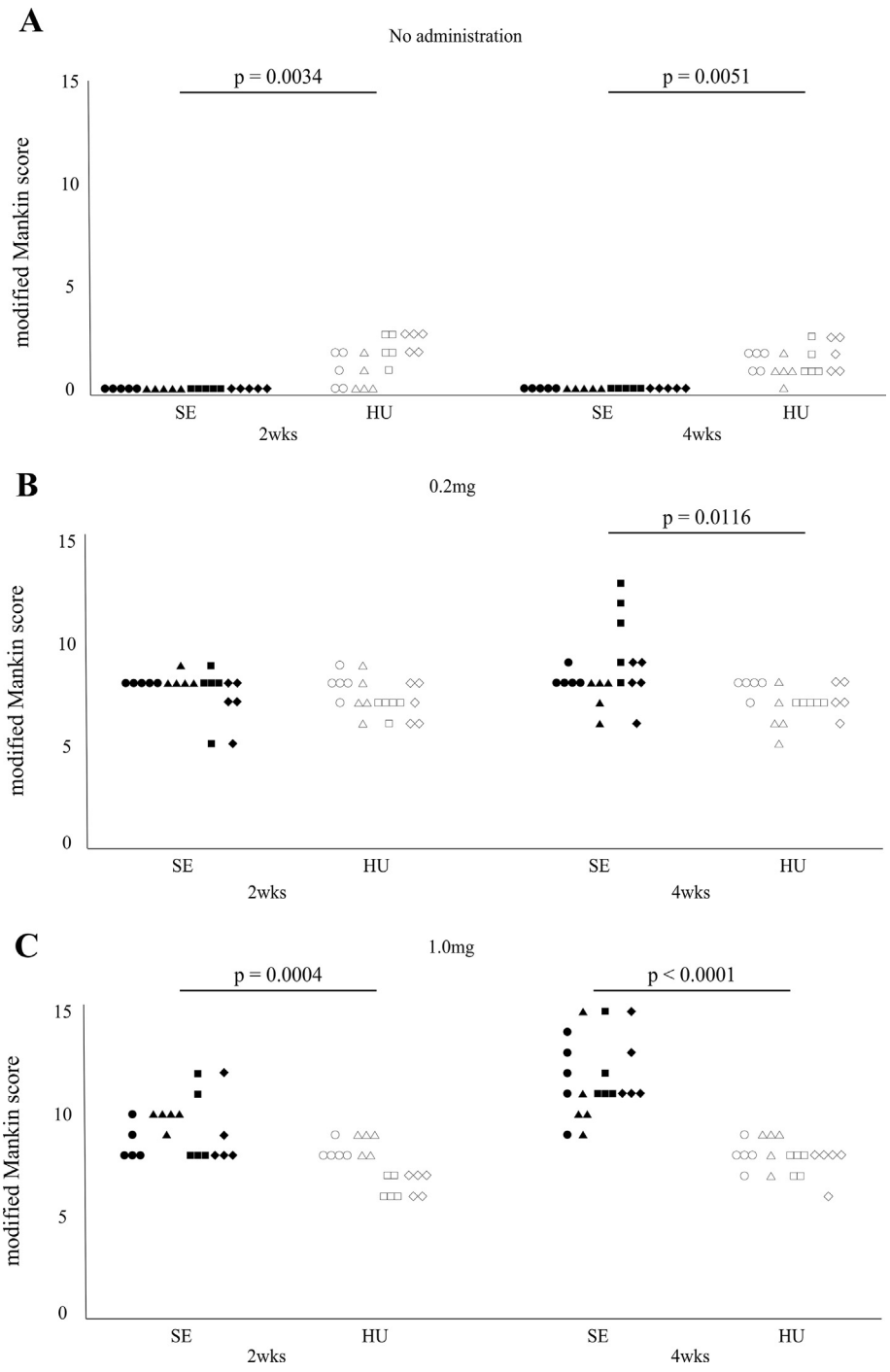

Fig. 6. Time-course changes in the modified Mankin score of no (A), 0.2 mg (B), and 1.0 mg (C) MIA administration. There were statistically significant intergroup differences in the modified Mankin score at 2 and 4 weeks of no MIA administration ($P = 0.0034$ and 0.0051, respectively). Following 0.2 mg MIA administration, a significant difference was noted in the modified Mankin score at 4 weeks ($P = 0.0116$). Additionally, after 1.0 mg MIA administration, a significant difference in the modified Mankin score was noted at 2 and 4 weeks ($P = 0.0004$ and $P < 0.0001$, respectively). Each shape indicates each specimen score; black and white shapes indicate the SE and HU groups, respectively. The circle, triangle, square, and rhombus indicate the score of the patella and femur in the PF joint and tibia and femur in the TF joint, respectively.

chondrocytes[44]. Based on this data, we assumed that there existed a difference in histological changes between the PF and TF joint in the present study and that there was a possibility that the site-specific difference could not be detected because of a small number of samples.

The MIA model has been established as an OA model and is known to exhibit a rapid progression rate. To perform hindlimb suspension, we had to shorten the experimental period; therefore, we opted for the MIA model as a model of OA. As a result, our data of the histological findings and scores suggested that the unloading condition suppresses articular cartilage degeneration. Although the MIA model is not related to the etiology of human OA, our study findings provide new histological evidence and are beneficial in many areas of basic and clinical researches involving OA. Further researches are required for investigating the effects of unloading condition on articular cartilage degeneration using the surgical

model of OA by various analyses, including immunohistochemistrical, histomorphometric, and biomechanical analyses.

Authors' contributions

All of the authors made substantial contributions to: (1) the conception and design of the study, data acquisition, and analysis and interpretation of the data; (2) drafting the article or its critically revision for important intellectual content; and (3) the final approval of the submitted version of the manuscript.

The authors' specific contributions were as follows:

(1) Conception and design of the study: IT, TM, HK, and MH
(2) Analysis and interpretation of the data: IT, TM, HK, and MH
(3) Drafting of the article: IT, HK, and MH
(4) Critical revision of the article for important intellectual content: IT, HK, and MH
(5) Final approval of the article: IT, TM, HK, and MH
(6) Obtaining funding: IT, TM, HK, and MH
(7) Collection and assembly of the data: IT, TM, and MH

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Competing interest statement

The authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Supplementary data

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