Osteoarthritis and Cartilage

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Monoiodoacetic acid induces arthritis and synovitis in rats in a dose- and time-dependent manner: proposed model-specific scoring systems

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S U M M A R Y

Objective: In a rat monoiodoacetic acid (MIA)-induced arthritis model, the amount of MIA commonly used was too high, resulting in rapid bone destruction. We examined the effect of MIA concentrations on articular cartilage and infrapatellar fat pad (IFP). We also established an original system for "macroscopic cartilage and bone score" and "IFP inflammation score" specific to the rat MIA-induced arthritis model.
Design: Male Wistar rats received a single intra-articular injection of MIA in the knee. The amount of MIA was 0.1, 0.2, 0.5, and 1 mg respectively. Articular cartilage was evaluated at 2–12 weeks. IFP was also observed at 3–14 days.
Results: Macroscopically, low MIA doses induced punctate depressions on the cartilage surface, and cartilage erosion proceeded slowly over 12 weeks, while higher MIA doses already induced cartilage erosion at 2 weeks, followed by bone destruction. MIA macroscopic cartilage and bone score, OARSI histological score, and Mankin score increased in a dose- and time-dependent manner. The IFP inflammation score peaked at 5 days in low dose groups, then decreased, while in high dose groups, the IFP score continued to increase over 14 days due to IFP fibrosis.
Conclusions: Punctate depressions, cartilage erosion, and bone destruction were observed in the MIA-induced arthritis model. The macroscopic cartilage and bone scoring enabled the quantification of cartilage degeneration and demonstrated that MIA-induced arthritis progressed in a dose- and time-dependent manner. IFP inflammation scores revealed that 0.2 mg MIA induced reversible synovitis, while 1 mg MIA induced fibrosis of the IFP body.

Introduction

Osteoarthritis (OA) is one of the most prevalent joint diseases, especially in women[1]. To explore the pathological mechanisms behind OA and develop new treatments, it is essential to establish optimal OA animal models. Inflammation as well as mechanical stress triggers OA and affects its progression[2–4]. Monoiodoacetic acid (MIA) is commonly used to induce arthritis in rats, primarily in studies of arthritis-related pain[5,6]. However, in most cases the large amount of MIA for these studies resulted in bone destruction beyond cartilage inflammation within a short period of time[5,7,8]. We made a hypothesis that low dose of MIA induced mild inflammatory features of the cartilage. The first aim of this study was to describe cartilage characteristics during the progression of arthritis induced by various amounts of MIA in rats.

To properly evaluate arthritis progression, a quantitative evaluation tool to describe whole features is required to compare

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disease progression in numerous specimens across multiple studies. For this reason, there are several scoring systems to evaluate cartilage degeneration macroscopically in each study that used arthritis models[7,9–12]. However, cartilage inflammation can vary with each model, especially in its early phase, and current scoring systems may not be precise enough to fully categorize each phase of disease progression. The second aim of this study was to establish an original scoring system to quantify arthritis of the knee joint specifically for the rat MIA-induced arthritis model.

Synovitis is an important pathological condition in arthritis and OA, and the infrapatellar fat pad (IFP) is a useful tissue to evaluate synovitis. There are some scoring systems to evaluate synovitis[13,14], however, these systems only evaluate the cell lining layers, ignoring the body of the IFP, though fibrosis of the IFP is an important pathological condition related to synovitis[15]. The third aim of this work was to establish an IFP inflammation score capable of evaluating both the surface and body of the IFP and to quantify synovitis in the rat MIA-induced arthritis model.

Materials & methods

Animals

This study was approved by the Animal Committee of Tokyo Medical and Dental University. All animal care and experiments were conducted in accordance with the institutional guidelines of our Animal Committee. One hundred and twenty male Wistar rats (Charles River, Japan) at 8 weeks of age, 270–285 g in weight, were used for the study.

Preparation of MIA arthritis model for cartilage evaluation

Monosodium iodoacetate (Sigma–Aldrich, St. Louis, MO) was dissolved in saline and used as MIA. Under anesthesia by isoflurane inhalation and intraperitoneal injection of tribromoethanol, the right knee joint had a single intra-articular injection of MIA in 50 μl of sterile saline. The dose of MIA was 0.1, 0.2, 0.5, or 1.0 mg/50 μl and 20 rats were used for each dose. The left knee joint received an injection of saline. The left knee of rats which had a 0.1 mg injection in the right knee was used as control. The knee fixed at 90° and MIA or phosphate buffer saline (PBS) was injected through patellar tendon. After the injection, the animals were returned to their cages and kept under a 12/12 h light/dark cycle with food and water.

Macroscopic observation

The knee joints were harvested at 2, 4, 6, 8, and 12 weeks after injection (Fig. 1). The tibial plateau was carefully separated from the femoral condyle. Macroscopic pictures of the femoral and tibial condyles were taken using a ZIESS Stemi 2000C microscope (Zeiss, Oberkochen, Germany) on a dedicated medical photography
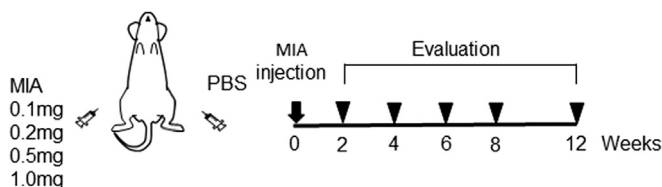
Fig. 1. Study schema. Rats had a single monoiodoacetic acid (MIA) injection in the right knee and phosphate buffer saline (PBS) in the left knee. The knees were evaluated at 2, 4, 6, 8 and 12 weeks after injection. For the control, the left knee of rats which had a 0.1 mg injection in the right knee, was used.

platform. Quantification of the size of the cartilage legion was performed using AxioVision Rel 4.8 software (Zeiss). The cartilage degeneration and bone destruction of the femoral and tibial condyles were evaluated using a macroscopic score on a scale of 0–5 points (Table I).

Histological examination

Proximal tibias were fixed in 4% paraformaldehyde for 7 days, decalcified in 20% EDTA solution for 21 days, and then embedded in paraffin wax. The specimens were sagittally sectioned at 5 μm and stained with safranin-o and fast green. Histological sections were visualized using an Olympus BX53 microscope (Olympus, Tokyo, Japan). The cartilage degeneration of the medial tibial plateau was evaluated using OARSI score[16] on a scale of 0–24 points and Mankin score on a scale of 0–14 points[17].

Immunostaining

Paraffin-embedded sections were deparaffinized in xylene, rehydrated in graded alcohol, and washed in PBS. All subsequent incubations were performed in a humidified chamber. The section was pretreated with proteinase K (Dako, Glostrup, Denmark) in Tris HCl buffer for 15 min at room temperature for optimal antigen retrieval. Then endogenous peroxidases were quenched using 0.3% hydrogen peroxidase in methanol for 15 min. Primary antibodies for human anti-type II collagen (Kyowa Pharma Chemical, Toyama, Japan) were applied to sections and incubated at room temperature for 1 h. After extensive washes with PBS, the sections were incubated in the biotinylated horse anti-mouse IgG for type II collagen. Immunostaining was detected with Vectastain ABC regent (Vector, Burlingame, CA) followed by diaminobenzidine staining. The sections were counterstained with hematoxylin.

Evaluation of synovitis

For the evaluation of synovitis, 40 rats were divided into two groups and 0.2 or 1.0 mg of MIA in 50 μl of sterile saline was injected into the right knee joint. The whole knee joint was harvested at 0, 3, 5, 7, and 14 days after the injection and prepared for histological evaluation as described previously (Fig. 5). The slides were stained with hematoxylin and eosin (HE), and the synovitis was evaluated using the IFP inflammation score on a scale of 0–6 points (Table II).

Statistical analysis

The scores were evaluated by three independent observers, where two observers evaluated in a blinded manner. For histology, one representative slice was evaluated. Interclass correlation

Table I
Macroscopic cartilage and bone scoring (0–5) for rat arthritis induced by mono-iodoacetic acid (MIA)

Points	Findings
0	Intact articular surface
1	≦10 punctate depressions per condyle*
2	>10 punctate depressions per condyle*
3	Erosion (≦50% of joint surface)
4	Erosion (>50% of joint surface)
5	Bone destruction

Both lateral condyle and medial condyle were evaluated separately, and the higher point value was selected for femur and tibia respectively.

* Condyle; lateral femoral condyle, medial femoral condyle, lateral tibial condyle or medial tibial condyle.

Table II
Infrapatellar fat pad (IFP) inflammation scoring (0–6)

Points	Histological signs
Cell infiltration at the surface of the IFP	
0	Normal
1	Cellularity is increased, multinucleated cells present
2	Thickened lining cells, low (<threefold thickness of the normal synovium)
3	Thickened lining cells, high (>threefold thickness of the normal synovium)

| Points	Histological signs
Fibrosis in the body of the IFP	
0	No fibrotic lesion
1	Fibrotic lesion in infrapatellar fat-pad present, low
2	Fibrotic lesion is increased, high
3	Infrapatellar fat-pad filled with the fibrotic lesion and fat cells absent

coefficients (ICC) for the inter observer variability among three observers were 0.948 (macroscopic score), 0.975 (histological score), and 0.962 (synovitis score). The scores evaluated by single observer were demonstrated.

The sample size was four for each group. The analysis unit was a group dependent of MIA dose in each period. The non-parametric Steel–Dwass test was performed using StatView 5.0 (SAS Institute, Cary, NC) for the analyses of macroscopic score, OARSI score, Mankin score and synovitis score. *P* values less than 0.05 were considered significant.

Results

Macroscopic observation of tibial cartilage

In the control group, cartilage in the left knee of rats, which had a 0.1 mg injection in the right knee, was not affected over 12 weeks [Fig. 2(A)]. No cartilage lesion was observed in the first 4 weeks in the 0.1 mg group. Some punctate depressions were first observed at 6 weeks. These were more apparent after India ink staining [Fig. 2(B)]. Punctate depressions enlarged but cartilage surface was still glossy at 8 weeks. The number of punctate depressions increased at 12 weeks. In the 0.2 mg group, some punctate depressions were already observed at 4 weeks. The number of punctate depressions increased at 6 weeks. Cartilage erosion was observed at 8 and 12 weeks. In the 0.5 mg group, cartilage erosion was already observed at 2 weeks, and occupied most of the surface at 4 weeks. Bone destruction emerged
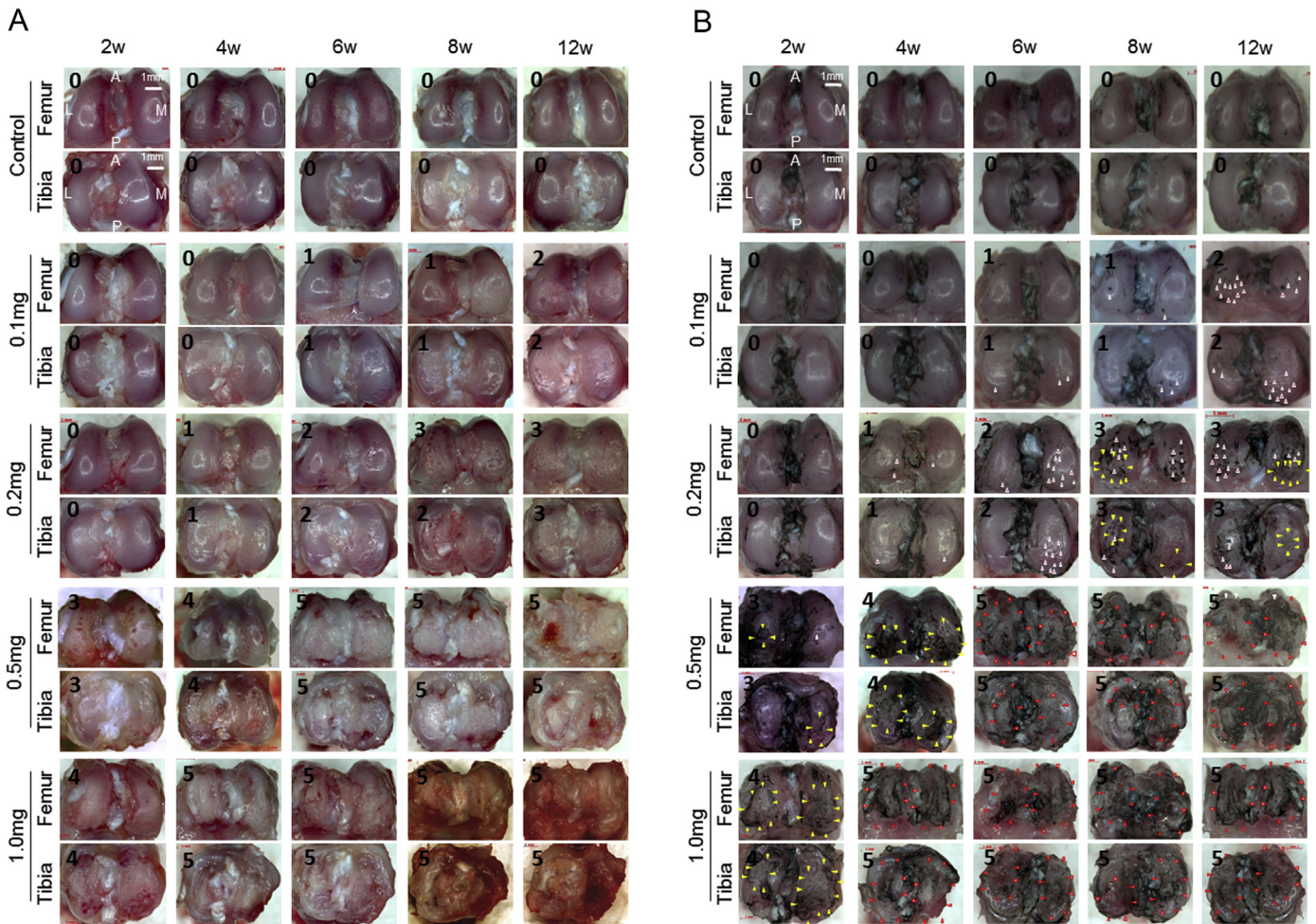
Fig. 2. Representative macroscopic features of the femoral and tibial condyle cartilage. (A) Images without India ink staining. Macroscopic cartilage score introduced in Table I is shown in each image. The tibial image with median value is selected among four samples and the femur is selected from the same knee. In the control group, the images of the left knee were inverted horizontally to match the images in the other groups. (B) Images with India ink staining. Punctate depressions are indicated with white arrowheads. Erosions are surrounded with yellow arrowheads. Areas of bone destruction are surrounded with red arrowheads. A; anterior, P; posterior, M; medial, L; lateral.

at 6 weeks and progressed at 8 and 12 weeks. In the 1 mg group, cartilage erosion occupied most of the articular surface at 2 weeks. Bone destruction was evident at 4 weeks and thereafter. The macroscopic features of femoral cartilage appeared to be similar to those of tibial cartilage. In the control group which had 0.2 mg, 0.5 mg or 1 mg injection in the right knee, was not affected over 12 weeks (Images were not shown).

Quantification of macroscopic features for tibial cartilage

To quantify the macroscopic features of cartilage from the knee, we established a macroscopic cartilage and bone score specific for rat arthritis induced by MIA (Table I). In the 0.1 mg group, the score for the tibial cartilage was 0 at 2 weeks, and then gradually increased thereafter. In the 0.2 mg group, the score was 0.3 at 2 weeks, increased gradually at 4 and 6 weeks, and reached a plateau from 8 to 12 weeks. In the 0.5 mg group, the score was 2.8 at 2 weeks, increased rapidly at 4 weeks, gradually increased at 6 and reached the maximum score of 5 from 8 to 12 weeks. In the 1 mg group, the score was 3.8 at 2 weeks, and then reached to the maximum score by 4 weeks on. The scores for femoral cartilage showed similar trends to the scores for tibial cartilage (Fig. 3).

Histological observations of tibial cartilage

In the control group, cartilage evaluated by safranin-o staining was not affected over 12 weeks [Fig. 4(A)]. In the 0.1 mg group, cartilage matrix staining slightly decreased at 2 weeks, and then continued to decrease thereafter [Fig. 4(A)], though cartilage matrix evaluated by type II collagen immunostaining was not similarly affected (Supplementary Fig. 1). In the 0.2 mg group, staining decreased moderately at 2 weeks, and was lost to the deep zone at 4 weeks and thereafter, though remaining cartilage matrix was still positive for type II collagen (Supplementary Fig. 1). In the 0.5 mg and 1 mg groups, subchondral bone was already exposed at 2 weeks and began eroding at 4 weeks and thereafter.

Quantification of histological features of tibial cartilage

Histological features for tibial cartilage were quantified by OARSI score [Fig. 4(B)]. In the 0.1 mg group, the score was 3.5 at 2 weeks, and then gradually increased thereafter. In the 0.2 mg group, the score was 5.5 at 2 weeks, then rapidly increased at 4 weeks, and remained relatively unchanged thereafter. In the 0.5

and 1.0 mg groups, the scores were 19 and 20 at 2 weeks, and then reached to the maximum of 24 at 4 weeks. Histological features were also quantified by Mankin score, which showed similar trends to that of OARSI score [Fig. 4(C)].

IFP inflammation

To evaluate synovitis, 0.2 mg or 1 mg MIA was injected and the IFP was histologically evaluated [Fig. 5(A)]. In the 0.2 mg group, the thickness of lining cells at the surface of the IFP increased and fibrosis was observed in the body of the IFP at 3 days [Fig. 5(B)]. The fibrosis area enlarged at 5 days, but decreased at 7 days. The thickness of lining cells decreased at 14 days. In the 1 mg group, synovitis similar to that in the 0.2 mg group was observed at 3 and 5 days respectively. The thickness of lining cells further increased at 7 days, and fibrosis occupied the entire body of the IFP at 14 days.

To quantify inflammation, an IFP inflammation score was established (Table II). This IFP inflammation score consisted of "Cell infiltration at the surface of the IFP" and "Fibrosis in the body of the IFP". The IFP inflammation scores increased at 3 and 5 days [Fig. 5(C)]. The score then decreased in the 0.2 mg group, but increased further in the 1 mg group at 7 and 14 days.

Discussion

In this study, 0.1 mg MIA induced punctate depressions at 6 weeks but did not induce erosion until 12 weeks. At 0.2 mg, MIA induced punctate depressions at 4 weeks and erosion at 8 weeks. At 0.5 mg and 1 mg, MIA induced bone destruction in as early as 4 weeks. MIA-induced arthritis progression was dose- and time-dependent. These results provide valuable information for the standardization of joint inflammation induced by MIA.

One of mechanisms by which MIA induces arthritis is by decreasing proteoglycan content. We demonstrated that cartilage matrix stained by safranin-o already decreased at 2 weeks after even with the 0.1 mg MIA injection, though cartilage matrix immunostained by type II collagen was not affected. Another mechanism of MIA action is reduced chondrocyte metabolism and induced death of chondrocytes by inhibition of the glycolytic system[7].

There have been several previous reports demonstrating the inflammatory morphology of knee joints induced by MIA in Wistar rats. Some papers observed results similar to ours. Mohan *et al.* observed cartilage degeneration at 10 weeks after the injection of
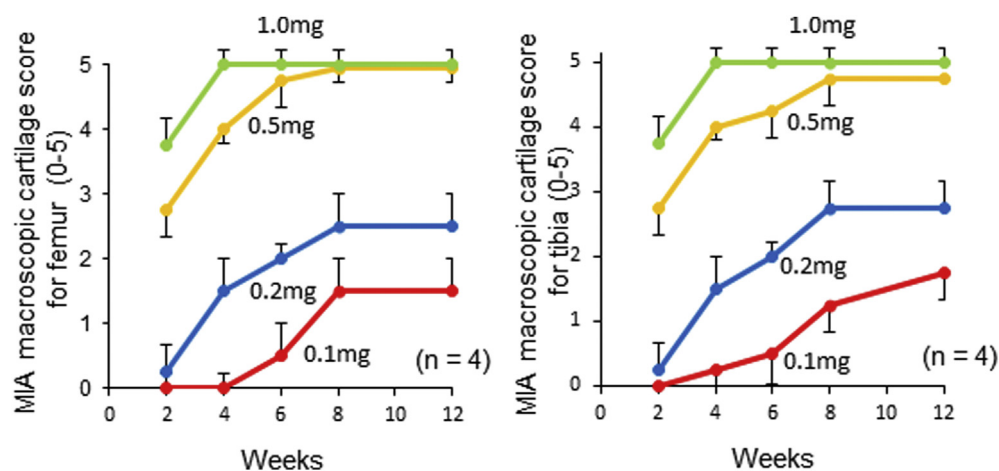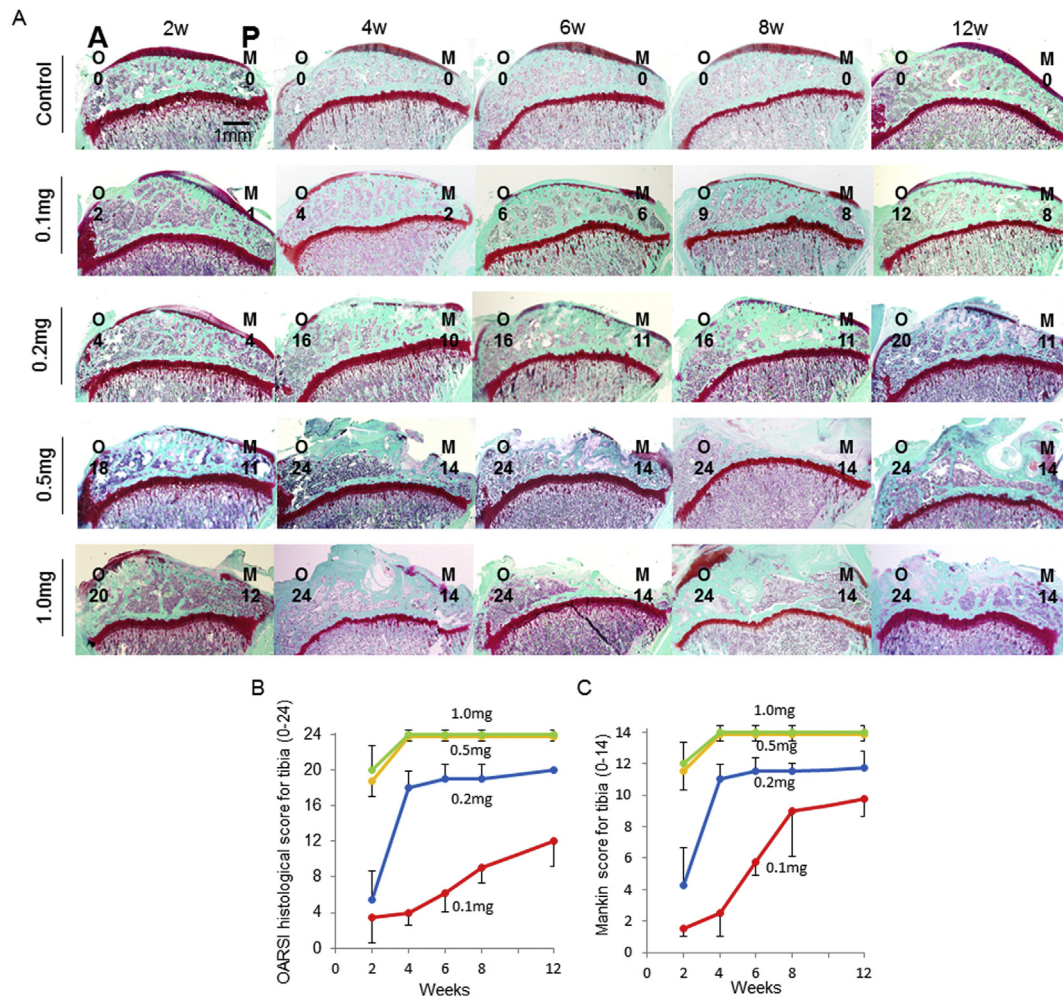

Fig. 3. Macroscopic cartilage score for femoral condyle and tibial plateau. Values are means with 95% confidence interval (*n* = 4).

Fig. 4. Histological analyses for the tibial condyle. (A) Representative histological features of the tibial condyle. Medial tibial condyle was sectioned in the sagittal plane and stained with safranin-o. OARSI histological score and Mankin score are shown in each image. The tibial plateau image with median value is demonstrated. A; anterior, P; posterior, O; OARSI histological score, M; Mankin score. (B) OARSI histological score for tibial cartilage. Values are means with 95% confidence interval ($n = 4$). (C) Mankin score for tibial cartilage.

0.2 mg MIA. The macroscopic and histological findings of cartilage degeneration were similar to ours and OARSI histological scores were almost equal to ours[18]. Thote *et al.* induced cartilage degeneration by 0.2 mg MIA and showed exposure of subchondral bone at 3 weeks[19], which was similar to ours. Clemants *et al.* reported subsynovial fibrosis in infrapatellars fat pads 21 days after 1 mg MIA injection[16], again, results similar to ours.

Other previous papers showed different results from ours, in part or as a whole. Guingamp *et al.* reported that the cartilage at 30 days after injections of 0.1 mg MIA looked similar to ours, however, the bone destruction was not observed even after injection of 3 mg MIA[8]. Ferreira-Gomes *et al.* showed that 0.3 mg MIA induced cartilage degeneration at 31 days[17] which was similar to ours, however, 1 mg and 2 mg MIA did not induce bone destruction even at 4 weeks, which was different than ours. Bove *et al.* reported the absence morphological changes of the knee joint induced by 1 mg MIA after 7 days[5]. The variation in the results of these three papers and in ours could likely be attributed to the different rat body weights; rats used in these three papers were lighter, suggesting younger rats were used. Wistar rats were used in this study and the effects of MIA in the knee joint may differ between rat strains. Mapp *et al.* injected 1 mg MIA into Sprague–Dawley rats and observed less bone destruction though the same amount of MIA was used[19,20]. Even the 2 mg or 3 mg MIA did not induce bone destruction[19,21]. Thakur *et al.* and Pomonis

et al. compared histopathological findings of different doses of MIA in Sprague–Dawley rats. The doses were effective, however, the cartilage degeneration looked milder than that of 1 mg MIA in Wistar rats[22,23]. Given the results from our findings, the differences between strains must be noted when applying these data.

The amount of MIA administered and time after MIA injection were important factors affecting the severity of arthritis. Though we did not identify how long MIA remained in the knee joint, histological features for cartilage degeneration progressed for at least 12 weeks, even after injection of 0.1 mg MIA. This indicates that even a low amount of MIA is sufficient to produce cartilage degeneration without bone destruction, which is similar to the pathology of OA. Future animal models could be developed using lower concentrations of MIA over longer periods of time that may also benefit from the scoring system. To quantitate the macroscopic features for rat MIA arthritis models, Guingamp *et al.* previously reported a simple scoring system, a scale of 0–4 of increasing severity (0 = normal; 4 = maximum severity)[7], which was also used by others[12,24]. This system lacks the complexity to quantitate and describe many of the macroscopic features of cartilage degeneration induced by MIA.

We included punctate depressions in our macroscopic cartilage and bone scoring system. Punctate depressions are dotted erosive cartilage lesions, approximately 0.1 mm in diameter, observed in
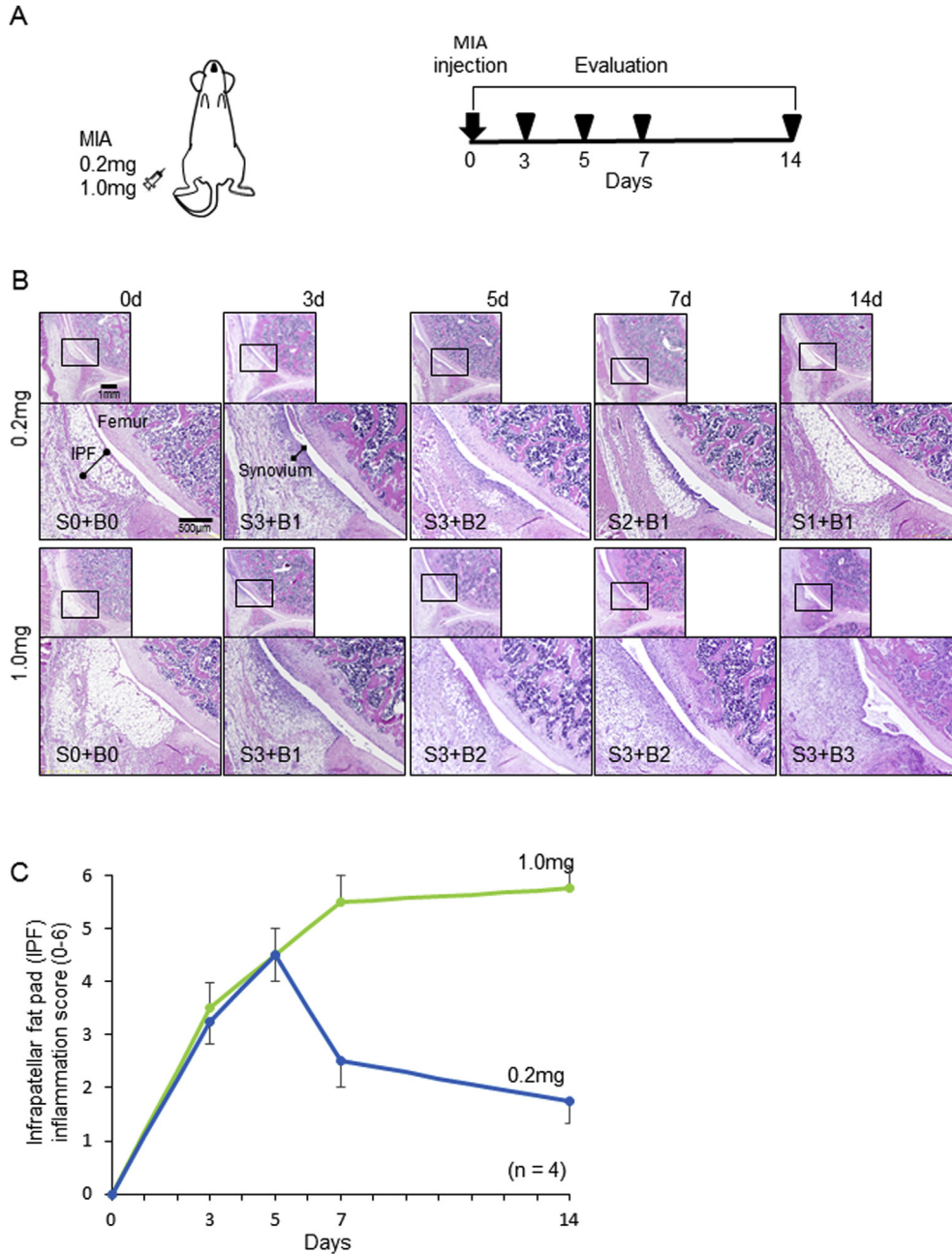
Fig. 5. Analysis for infrapatellar fat pad (IFP). (A) Study schema. (B) Representative histological images for the IFP. Whole knee joints were sectioned in the sagittal plane stained with hematoxylin and eosin. Infrapatellar fat pad inflammation score (surface score + body score) are shown in each image. (C) Infrapatellar fat pad inflammation score. Values are means with 95% confidence interval ($n = 4$).

arthritis specifically induced by MIA. In this study, punctate depressions were demonstrated in rats but may be observed in other animals as well. We observed punctate depressions in the figures of a previous paper where MIA was injected into the knee of Wistar rats, where the punctate depressions were termed focal erosions[7]. In another paper, MIA was injected into the knee of Sprague–Dawley rats, and punctate depressions could be also observed but were termed "mild damage"[10]. We defined punctate depressions in our paper for the first time. We set 10 punctate depressions per condyle as the cut off between one and two points in the macroscopic cartilage and bone scoring system, because at that cutoff, the number of samples with each score in the MIA 0.1 and 0.2 mg groups were nearly identical.

OARSI histological scores and Mankin scores reached their maximum values by 4 weeks after 0.5 or 1 mg MIA injections. These dosages of MIA are too high to evaluate cartilage, because they resulted in not only cartilage inflammation, but also rapid bone destruction. To examine the effects of drugs and surgical interventions on modifying arthritis progression, MIA injections greater than 0.5 mg would be inappropriate to evaluate by histological scoring after 4 weeks.

For histological analysis, we evaluated the medial tibial condyle and not the lateral tibial condyle, because macroscopic cartilage and bone scores were higher for the medial condyle than the lateral condyle in the majority of samples. In other papers reporting knee inflammation induced by MIA in Wistar rats, only the medial

condyle was demonstrated histologically[25]. There are several reports showing that load distribution is higher on the medial side of the femorotibial joint than on the lateral side in rats[20,21,26].

Synovitis is one of the typical features of arthritis including OA[2] and in clinical pathology, and synovitis can adversely affect joint function[27]. There were no obvious differences in inflammation of the IFP between in the 0.2 mg MIA group and in the 1 mg MIA group at 3 and 5 days. After 5 days, inflammation of the IFP was reduced in the 0.2 mg group but progressed in the 1 mg group at 7 and 14 days. This suggests that low amounts of MIA induce reversible inflammation of the IFP, while high amounts of MIA induce sustained inflammation of the IFP.

The injection of 1 mg MIA induced fibrosis of the body of the IFP. Though an important finding, this was not reflected by the previous synovitis scoring system[13]. To quantitate the fibrosis in the body of the IFP, we established the IFP inflammation score, which grades "cell infiltration at the surface of the IFP" and "fibrosis in the body of the IFP". This scoring system could enable the clarification of temporal and sustained inflammation of the IFP.

We examined only two doses of MIA in contrast to the evaluation of articular cartilage at four doses. According to previous reports[7,18,28,29] and ours, we regarded 0.2 mg MIA as the representative of a low dose MIA model and 1 mg MIA as the representative of a high dose MIA model. For this study, we did not prepare a control for which the effect of injury caused by inserting a needle with saline into the knee joint was tested. One final consideration is that here we used Wistar rats at 8 weeks of age. The growth plate of 8 weeks old rats remained open, indicating possibility of skeletal immaturity. However, 7–8 weeks old rats are sexually mature[30]. There are many published papers in which Wistar rats at 8 weeks old and younger were used for arthritis induced by MIA[5,18,28,29]. The use of rats at 8 weeks of age is popular for arthritis models induced by MIA.

In this study, MIA-induced degenerative changes of cartilage and bone were time- and dose-dependent. Also, inflammatory changes of the IFP were reversible in the 0.2 mg MIA group, but irreversible in the 1.0 mg MIA group. When an MIA-induced arthritis model is used for a specific study, MIA dose and observation period should be carefully considered to suit the intended study duration and pathology. The proper application of MIA for rats could provide a useful tool to help determine the safety and efficacy of future arthritis treatments.

Conclusions

Low dose MIA induced punctate depressions on the surface of cartilage at 0.1 mg and 0.2 mg, and cartilage erosion proceeded with time, in Wistar rats. Higher doses at 0.5 mg and 1 mg MIA induced bone destruction at 4 weeks. We established a macroscopic cartilage and bone scoring system that enabled facile quantification of cartilage degeneration and demonstrated that MIA-induced arthritis progression is dose- and time-dependent. An IFP inflammation score revealed that 0.2 mg MIA induced reversible synovitis, while 1 mg MIA induced irreversible synovitis. Taken together these data present a compelling new use for low MIA dosing to study arthritis in pre-clinical animal models.

Author contributions

Mio Udo: Conception and design, collection of data, data analysis, and manuscript writing. Takeshi Muneta: Conception and design, administrative support. Kunikazu Tsuji: Conception and design, technical support. Nobutake Ozeki: Conception and design, collection of data. Yusuke Nakagawa: Conception and design, collection of data. Toshiyuki Ohara: Conception and design, collection of data. Ryusuke Saito: Conception and design, collection of data, data analysis. Katsuaki Yanagisawa: Conception and design, collection of data, data analysis. Hideyuki Koga: Conception and design, interpretation of data. Ichiro Sekiya: Conception and design, financial support, manuscript writing, final approval of manuscript.

Conflict of interest

No conflict of interest for any of the authors.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2016.02.005.

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