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Original Research Article

Concordance of Radiological and Histopathological Features in a Monosodium Iodoacetate–Induced Osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is a common degenerative joint disease characterized by progressive impairment of joint function involving cartilage and synovium. Although histopathological examination remains the gold standard for confirming OA, radiological assessment in small-animal models is less frequently reported. In this study, we evaluated the concordance between radiological and histopathological OA features in a rat model induced by intra-articular injection of monosodium iodoacetate (MIA). Twenty male Wistar rats were divided evenly into four groups (Control, P1, P2, P3), with the latter three groups receiving 2 mg/kg of MIA in the right knee. Radiological (Kellgren–Lawrence) and histopathological (OARSI) scores were assessed at days 7 (P1), 14 (P2), and 21 (P3). Compared to the control, both K&L and OARSI scores were significantly elevated on days 14 and 21 ($p < 0.001$). Radiological and histological features showed good concordance over time, revealing clear, progressive OA-like changes by day 21. Our study revealed that the histopathology of features in experimental OA by using MIA has significant concordance with the radiographic manifestation.

Keywords: Osteoarthritis, K&L Score, Oarsi Score, Monoiodoacetate, Rat Model

Introduction

Osteoarthritis (OA) is a degenerative joint disease commonly encountered worldwide, and its incidence increases with age[1]. It is characterized by joint pain and a progressive decline in joint function, influenced by pathological changes in the bone, cartilage, and synovium [2]. Globally, OA is among the leading causes of disability related to joint pain. In many patients, pain is brought on by daily activities, which limits mobility and reduces quality of life [3]. The diagnosis of OA typically relies on clinical signs such as bone enlargement, joint pain, crepitus, and joint stiffness, followed by radiological imaging [4]. In some instances, arthroscopic examination may also be necessary [5]. The severity of OA in clinical settings is most often assessed via radiological changes using the Kellgren–Lawrence classification (grades 0–4), which focuses on joint space narrowing, osteophyte formation, and overall joint structure alterations [6]. However, direct evaluation of cartilage pathology via biopsy is rarely performed for OA diagnosis in humans because of the associated risks [7]. In experimental animals, on the other hand, OA severity can be assessed by both imaging and histopathological examination, the latter commonly using the Osteoarthritis Research Society International (OARSI) scoring system [8].

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The OARSI system evaluates changes in the cartilage matrix, chondrocyte morphology, cell death (apoptosis), and proteoglycan content [9]. Previous evidence from earlier research indicates a close realaionship between the generation of articular cartilage and modifications in subchondral bone architecture, evaluated through parameters such as bone volume, trabecular thickness, trabecular number, and trabecular fragmentation. It has been proposed that changes in subchondral bone are, at least in part, a consequence of cartilage breakdown [10].

As OA is multifactorial and diverse, various animal models have been developed to investigate its pathophysiology [11]. However, choosing the most appropriate model for a given research question can be challenging [12]. An ideal model should accurately mimic the disease and allow combined assessments using biomechanical, radiological, and microscopic methods. Existing OA induction strategies in animals include mechanical, surgical, and chemical methods, each with advantages and limitations [13].

One well-established approach involves injecting monosodium iodoacetate (MIA) to induce OA-like changes. MIA is a metabolic inhibitor that disrupts the aerobic glycolysis pathway by inhibiting glyceraldehyde-3-phosphate dehydrogenase in chondrocytes, ultimately leading to chondrocyte death [14]. Intra-articular MIA administration causes a decrease in chondrocyte numbers and structural changes in joint cartilage that resemble human OA [15]. Although MIA injection also affects the synovial membrane, assessing the joint histopathology requires sacrificing the animals, which is not feasible in studies requiring live models. Currently, there is limited information on whether radiological evaluations of OA in rats align closely with the histopathological changes in cartilage. Therefore, this study aimed to determine the concordance between radiological and histopathological findings of knee OA development induced by MIA in a rat model.

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Materials and Methods

Ethical Approval
All procedures were performed in accordance with guidelines approved by the Biosciences Laboratory of Universitas Brawijaya (No. 060-KEP-UB-2024).

Study Design
A total of 20 male Wistar rats (*Rattus norvegicus*), each weighing 200–250 g, were used in this study. The rats were housed in two per cage (30 × 40 × 20 cm) in an environment maintained at 25 °C with a 12-hour light–dark cycle. Food and water were provided *ad libitum*. The animals were randomly assigned to four groups (n = 5 per group). Control (C), injected with normal saline (NS) into the right knee; P1, injected with monosodium iodoacetate (MIA) at a dose of 2 mg/kg[5,7] into the right knee, observes at week 1; P2, injected with MIA 2 mg/kg), observed at week 2; P3, injected with MIA (2 mg/kg), observed at week 3.

OA Induction and Confirmation
Monosodium iodoacetate (MIA, cat#12512; Sigma, St. Louis, MO, USA) was dissolved in 25 µL of saline solution for a final dose of 2 mg per injection [12]. Intra-articular injections were administered dorsally into the right knee. The control group received only saline. Rats in each treatment group were subsequently examined at their designated time points (weeks 1, 2, or 3) for radiological and histopathological signs of osteoarthritis.

Radiographic examination
Radiographic images were obtained using a Shimadzu X-ray machine with an Agfa 24×30 cassette, which was marked into six equal sections to position the rat knees. Following disinfection, each rat's knee was gently extended and fixed in place under the X-ray beam. Exposure parameters (kV, mA, and time) were kept constant across all radiographs. All radiographs were interpreted by a Veterinary Radiology Consultant, who classified each knee joint according to Kellgren–Lawrence (K&L) grades 0–4[7]. This system assesses osteophyte formation, joint-space narrowing, subchondral sclerosis, and bony deformity. Additionally, the degree of joint-space narrowing was measured on the medial (right) and lateral (left) sides of the rat knees, and any visible structural changes were recorded.

Histopathological Examination

At each designated time point (day 7 for P1, day 14 for P2, and day 21 for P3), rats were euthanized and knee joints were harvested. The tissues were fixed in 10% neutral-buffered formalin for 24 hours. Next, samples underwent a standard dehydration series in graded ethanol, followed by clearing in xylene and impregnation with molten paraffin. Paraffin blocks were then sectioned at 4–5 µm using a rotary microtome. Slides were stained with hematoxylin and eosin (H&E). Examined the stained sections and assigned scores based on the Osteoarthritis Research Society International (OARSI) system [4]. This scoring ranges from 0 (no degeneration) to 5 (severe degeneration affecting >75% of the cartilage), capturing changes such as matrix erosion, chondrocyte loss, and proteoglycan depletion.

Data Analysis
Kellgren–Lawrence (K&L) and OARSI scores were presented as mean ± standard deviation (SD). One-way ANOVA was used to compare mean values across the four groups, followed by Tukey's HSD test for post hoc comparisons. All statistical analyses were conducted using GraphPad Prism (version 10). A p-value of < 0.05 was considered statistically significant.

Results and Discussion

In this study, rats injected with monosodium iodoacetate (MIA) exhibited typical osteoarthritis (OA) changes, including osteophyte formation, subchondral sclerosis, and joint-space narrowing. These changes became more noticeable over time, especially at weeks 2 and 3. As shown in Figure 1(A), the Kellgren & Lawrence (K&L) scores for panels A–D were 0, 1, 2, and 3, respectively, showing a clear progression of knee joint pathology with increased post-injection time. Figure 1(B) presents the statistical analysis of K&L scores across groups. No significant difference was found between the control and P1 groups (0.4 ± 0.5 vs. 0.8 ± 0.8; p = 0.73). In contrast, both the P2 group (2.4 ± 0.5) and the P3 group (3.8 ± 0.4) had significantly higher K&L scores than the control (p = 0.00 in each case), indicating noticeable radiographic changes from the second week onward. Joint-space narrowing (JSN) was also measured bilaterally (Figure 1(C). On the right side, there were no significant differences between the control and P1 groups (0.11 ± 0.02 mm vs. 0.10 ± 0.05 mm; p = 0.95), nor between the control and P2 (0.07 ± 0.02 mm; p = 0.32) or P3 (0.08 ± 0.03 mm; p = 0.62). Similar findings were seen on the left side, with no significant changes in JSN among the control (0.06 ± 0.05 mm), P1 (0.07 ± 0.02 mm), P2 (0.05 ± 0.03 mm), and P3 (0.12 ± 0.03 mm) groups. Taken together, these results suggest that while osteophytes and sclerosis emerged clearly by weeks 2 and 3, consistent joint-space narrowing was less pronounced in this OA model.

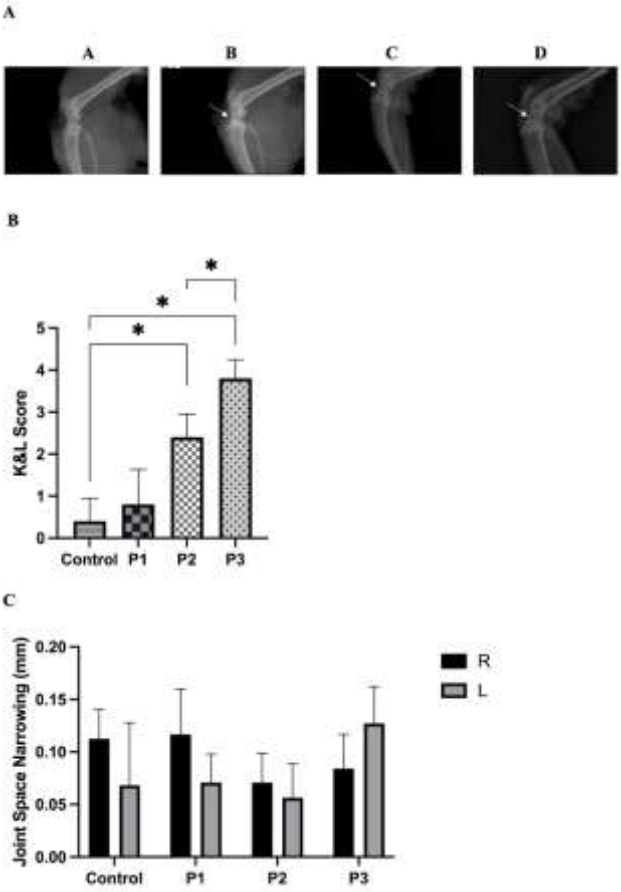

Figure 1: Radiological evaluation of MIA-induced osteoarthritis in knee joint tissues. (A) Representative radiographic changes showed a gradual progression of osteoarthritis: grade 1 at week 1 with mild inflammation and early joint changes (box B), grade 2 at week 2 with joint space narrowing, subchondral bone thickening, and small osteophytes (box C), and grade 3 at week 3 with larger osteophytes, joint deformity, and cartilage erosion (box D). (B) Statistical analysis of Kellgren–Lawrence (K&L) scores in control and MIA groups at day 7 (P1), day 14 (P2), and day 21 (P3), with significant differences indicated by ($p < 0.05$). (C) Quantitative statistical analysis of JSN. R right, L left. (Control) Radiological examination on day 7, (P1) radiological examination on day 7, (P2) radiological examination on day 14, and (P3) radiological examination on day 21.

In this study, radiological and histological changes in MIA-injected rats first appeared within the initial 7 days and continued to progress at days 14 and 21, following a time and dose-dependent pattern. At a dose of 2 mg/kg MIA, no significant difference in Kellgren–Lawrence (K&L) scores was observed between the control group and rats evaluated in the first week. By week 2, however, osteophytes, joint-space narrowing, and sclerosis became evident, corresponding to grade 2 changes, and by week 3 these features progressed to grade 3. Consistent with the radiographic observations, histopathological changes were detectable throughout the 21 days, although OARSI scores in the control and week 1 (P1) groups did not differ significantly. The week 2 (P2) group reached OARSI grade 2, and the week 3 (P3) group advanced to grade 3, illustrating the time-dependent escalation of joint damage.

These findings align with multiple prior studies examining MIA-induced knee inflammation in Wistar rats. For instance, Ise et al. compared 2 mg/kg and 0.5 mg/kg doses of MIA, demonstrating significant increases in OARSI and Larsen scores over time, coupled with CT findings that mirrored the histological results[10]. Studies have reported cartilage degeneration at 10 weeks post-injection of 0.2 mg/kg MIA; similarly, histological assessments in their study revealed subchondral bone exposure by 3 weeks[16]. Moreover, a low-dose (0.2 mg/kg) MIA model showed rapid synovitis resolution within 14 days, after which ongoing cartilage damage led to OA. This pattern contrasts with high-dose MIA models, where persistent synovitis has been noted beyond 14 days[17].

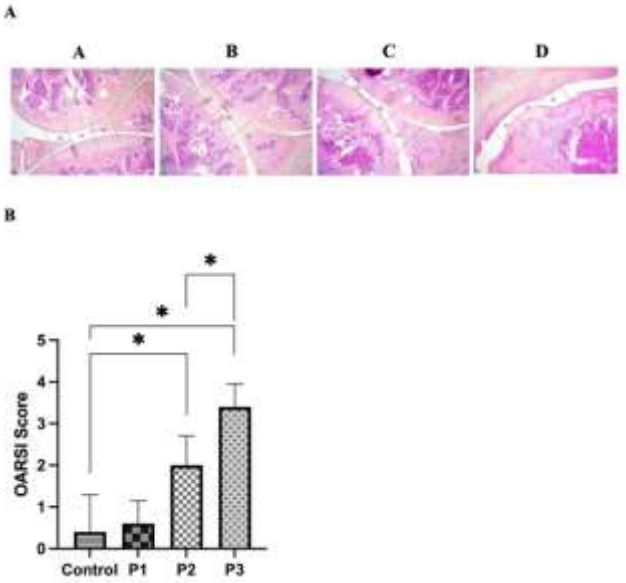

Figure 2: Histopathological evaluation of MIA-induced osteoarthritis in knee joint tissues. (A) Representative sections from control and MIA groups at week 1 (P1), week 2 (P2), and week 3 (P3). The control group shows normal cartilage with no degeneration (box A), while the MIA groups demonstrate progressive pathological changes, including early cartilage irregularities (P1), matrix loss and chondrocyte clustering (P2), and severe degeneration with cartilage erosion and joint architecture disruption (P3). (B) Statistical analysis of OARSI scores in control and MIA groups at day 7 (P1), day 14 (P2), and day 21 (P3). Significant differences are indicated by * ($p < 0.05$).

Mechanistically, MIA exerts inhibitory effects on glyceraldehyde-3-phosphate dehydrogenase (GAPDH), inducing chondrocyte death[16]. When administered intra-articularly in rodents and other species, MIA causes chondrocyte loss, matrix proteoglycan degradation, and functional stiffness reminiscent of human OA[18]. Yoh et al., 2022 further explored these processes in a rat hip OA model, showing that MIA-induced lesions exhibit both radiological and subchondral bone alterations over time, underscoring the relevance of MIA models for studying OA progression[18].

Radiological and histological assessments remain invaluable in animal models for the noninvasive, repeatable analysis of joint pathologies. Here, anteroposterior and lateral X-rays clearly illustrated how deformities of the femoral head and acetabulum developed over time, mirroring many aspects of the human disease[17]. Such radiographic evaluation can also help reduce the number of animals sacrificed, as it enables ongoing observation of OA progression without requiring repetitive tissue sampling[18].

In rat models of osteoarthritis (OA), regular walking exercise over six weeks at light intensity (10 meters/minute), medium intensity (15 meters/minute), and high intensity (20 meters/minute) leads to a lowered pain threshold, reflecting improved clinical outcomes. These changes are significantly correlated with decreased cytokine levels, suggesting that exercise reduces inflammation and subsequently alleviates disease symptoms [22,23].

Conclusion

In conclusion, intra-articular injection of 2 mg/kg monosodium iodoacetate (MIA) successfully induced progressive osteoarthritic changes in rat knee joints, as evidenced by both radiological (Kellgren–Lawrence) and histopathological (OARSI) assessments. The concordance between these two measures became especially clear from day 14 onward, supporting the validity of this model in capturing time-dependent OA pathology. These findings underscore the reliability of MIA-induced OA rats for studying the disease's progression and evaluating potential therapeutic interventions by using radiographic assessment.

Conflict of Interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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