Original Article

Rat Model of Lumbar Facet Joint Osteoarthritis Associated With Facet-mediated Mechanical Hyperalgesia Induced by Intra-articular Injection of Monosodium Iodoacetate

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Background/Purpose: The relationship between lumbar facet joint (LFJ) osteoarthritis (OA) and back pain remains unclear. An OA model associated with joint pain was successfully induced by monosodium iodoacetate (MIA) in rat knees. We aimed to establish an experimental OA model with facet-mediated mechanical hyperalgesia in the LFJ in rats.

Methods: We established a rat experimental model of LFJ OA with facet-mediated mechanical hyperalgesia by injection of MIA into a single facet joint. After injection, changes in the LFJ structure were assessed histologically, and mechanical hyperalgesia in the hind paw was determined using the von Frey test. In addition, interleukin-1β and tumor necrosis factor-α levels in the synovium were measured by enzyme-linked immunosorbent assay, and the inhibitory effects of celecoxib and gabapentin on mechanical hyperalgesia were evaluated.

Results: Progressive cartilage degeneration and changes in subchondral bone were observed after injection. A biphasic pattern of mechanical hyperalgesia was noted in the hind paw. The concentrations of interleukin-1β and tumor necrosis factor-α were significantly increased only on Days 1 and 3 when compared with controls. Celecoxib was effective only on Day 3 and ineffective on Days 21 and 35, whereas gabapentin kept its inhibitory effect on Days 3, 21 and 35.

Conclusion: An experimental LFJ OA model associated with facet-mediated mechanical hyperalgesia can be established by intra-articular injection of MIA. This model might provide a useful tool for further study to ascertain the complex mechanism of facet joint pain.

Key Words: animal model, lumbar facet joint osteoarthritis, monosodium iodoacetate, pain, rat
findings that support the role of OA of the facet joints as a cause of back pain were reported by Dolan et al., whereas Kailchman et al. demonstrated no connection between disease severity and the level of reported LFJ pain. However, these clinical studies have provided only a little useful information because human studies are very restrictive and the source of patients or specimens is limited. Further basic studies have been hampered because of the lack of a useful animal model.

Monosodium iodoacetate (MIA), an inhibitor of glyceraldehyde-3-phosphate dehydrogenase activity, can induce degenerative changes in the articular cartilage by direct interference with chondrocyte metabolism, and it has been successfully used to induce knee joint OA in rodents. In addition, it is interesting to find pain-related behavior changes in MIA-induced OA models. As only a few studies on LFJ OA models have been reported, we attempted to establish a new experimental model of LFJ OA in rats, associated with facet-mediated mechanical hyperalgesia induced by intra-articular injection of MIA.

Materials and Methods

All experimental procedures were approved by the Institutional Care of Experimental Animals Committee of the Fourth Military Medical University, and adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. Three-month-old male Sprague–Dawley rats, weighing 200–250 g, were housed under a 12-hour/12-hour light/dark cycle with free access to food and water.

Surgical procedure and intra-articular injection of MIA

Surgical procedures were performed under intraperitoneal anesthesia with 30 mg/kg sodium pentobarbital (Nembutal; 50 mg/mL, Sigma, Poole, UK). Rats were placed in a prone position and a 2-cm incision was made to the spinal midline. The fascia was incised along the right side of the supraspinous ligament for approximately 10 mm. The multifidus muscle was resected and the right L4/5 facet joint capsule was exposed under microscopy. MIA (Sigma) was dissolved in 0.9% sterile saline to provide the indicated concentrations (200 mg/mL). MIA (OA group, n = 121) or 0.9% sterile saline (control group, n = 49) was injected slowly into the right L4/5 facet joint at a final volume of 5 μL with a 36-gauge blunt NanoFil needle (WPI, Sarasota, FL, USA), at a rate of 2 μL/min, controlled by an infusion pump. After injection, the fascial layer of the muscles and the skin was sutured.

Histological study

On post-injection Days 3, 7, 14, 21, 28, 35 and 42, histological examinations were performed in the MIA and control groups (n = 3 on each day from each group). Animals were killed by cervical dislocation. The L4/5 spine was removed en bloc, fixed in 10% neutral buffered formalin for 48 hours, decalcified, and embedded in paraffin. Four randomly selected sections from each facet joint were obtained for toluidine blue staining. Degeneration of the facet joint was graded according to a modified Mankin’s grading system (total possible score = 26). Two independent examiners assessed the histological findings obtained from these specimens in a blind manner.

Behavioral test for mechanical hyperalgesia

Behavioral tests were conducted repeatedly before injection to obtain baseline thresholds for both groups, and then on postoperative Days 1, 3, 7, 14, 21, 28, 35 and 42 (n = 10 for each group). Animals were habituated to wire mesh bottom cages before the tests. Mechanical hyperalgesia was evaluated by application of von Frey hairs (Stoelting Instruments Inc., Wood Dale, IL, USA) in ascending order of force (0.7 g, 1.2 g, 1.5 g, 2 g, 3.6 g, 5.5 g, 8.5 g, 11.8 g, 15.1 g and 29 g) to the plantar surface of the hind paws. Each von Frey hair was applied to the paw for 6 seconds, and after a withdrawal response was established, the paw was retested, starting with the next descending von Frey hair, until no response occurred. The lowest amount of
force required to elicit a response was recorded as the paw withdrawal threshold (PWT) in grams.

Measurement of concentration of inflammatory cytokines
Synovium from the L4/5 facet joint was harvested on post-injection Days 1, 3, 7, 14, 21 and 42 \((n=3\) on each day for each group). A suspension was made by homogenizing the tissues in ice. After 30 minutes, centrifugal separation was carried out at 15,000 rpm at 4°C, and the supernatant was collected and measured. We measured interleukin (IL)-1β and tumor necrosis factor (TNF)-α by enzyme-linked immunosorbent assay, using the rat IL-1β EASIA kit (Huamei Biotech Inc., Wuhan, China) and rat TNF-α kit (Alpco Inc., Salem, New Hampshire, USA). The sensitivity was 3.12 pg/mL and 4 pg/mL, respectively. Each assay was performed in triplicate.

Pharmacological assessment
The inhibitory effects of celecoxib (Apin Chemicals, Abingdon, Oxon, UK; 30 mg/kg on Days 3, 21 and 42) and gabapentin (Apin Chemicals; 100 mg/kg on Days 3, 21 and 42) on mechanical hyperalgesia were investigated. Baseline PWT was taken before drug administration. Drugs or vehicle (5 mL/kg, 0.5% methyl cellulose) were administered orally in a volume of 1 mL. The PWT was assessed at 1, 2 and 4 hours after drug treatment \((n=6\) for each drug or vehicle on each day, total number = 72).

Statistical analysis
All data are presented as means ± standard deviation. For the mechanical threshold, the within group difference was analyzed using the Friedman test followed by the Wilcoxon signed-rank matched-pairs test. The difference between groups at a given time point was analyzed by the Mann–Whitney U test. For pathology score or cytokine concentration, one-way analysis of variance was used for comparison between two groups, and least significant difference t test was used for multiple comparisons between time points. A p value of <0.05 was set as the level of statistical significance for all tests.

Results
Degeneration of facet joint cartilage
In the control group, the cartilage did not degenerate at all predefined time points. The articular surface was smooth and the matrix was densely stained with toluidine blue (Figures 1A–D). In the MIA group, superficial fibrillation, reduction of toluidine blue in the superficial zone, and hypertrophic synovium intruding into the joint space were observed on Day 3 (Figure 1E). By post-injection Day 7, hypocellularity of chondrocytes in the superficial and mid zones, vertical fissures in the mid or deep zone, and reduction of toluidine blue staining in the deep zone were found (Figure 1F). By post-injection Day 21, cartilage erosion, focal matrix loss and exposure of subchondral bone were observed (Figure 1G). By post-injection Day 42, denudation of the articular cartilage, deformation of the articular surface, bone marrow fibrosis, and marginal osteophyte formation were observed (Figure 1H).

The modified Mankin’s scores are summarized in Figure 2. The scores differed significantly between the control and MIA groups at each time point \((p<0.05)\). In the saline control group, the scores were constant at a low level during the observation period. In the MIA group, the scores increased significantly with time \((p<0.05)\). However, the scores were no different between Days 35 and 42 \((p>0.05)\).

Behavioral study
The results of the behavioral test for mechanical hyperalgesia are shown in Figure 3. Animals in both groups showed stable conditions before surgery, in response to mechanical stimulation. A significant reduction in PWT was noted in the MIA group on Days 1, 3, 21, 28, 35 and 42 when compared with the control group \((p<0.05)\). In the control group, no different from baseline in PWT was found at all-time points \((p>0.05)\). In the MIA group, a biphasic pattern of change in PWT was observed. The first phase of decrease in PWT reached a peak on Day 3 and was followed by an increase on Days 7 and 14. On Days 7 and
Figure 1. (A–D) Histological changes of facet joints in rats after injection of monosodium iodoacetate or saline on Days 3, 7, 14, 21, 28, 35 and 42 ($n = 3$ at each day for each group). Normal articular cartilage was seen in saline controls during the observation period. (E) In the monosodium iodoacetate group, superficial fibrillation, reduction of toluidine blue in the superficial zone, and hypertrophic synovium intruding into the joint space were observed on Day 3. (F) On Day 7, hypocellularity of chondrocytes in the superficial and mid zones, vertical fissures (arrows) in the mid or deep zone, and reduction of toluidine blue staining in the deep zone were seen. (G) On Day 21, focal matrix loss, cartilage erosion, and exposure of subchondral bone (triangle) were observed. (H) On Day 42, denudation of the articular cartilage, deformation of the articular surface, bone marrow fibrosis (open arrows), and marginal osteophyte formation (asterisks) were observed.
14, there was no difference in PWT value between the control and MIA groups ($p > 0.05$). By post-injection Day 21, the threshold decreased again and reached its second trough on Day 35, and then kept constant up to Day 42. The change in PWT noted at the 35-day time point was not significantly different from that noted at 42 days post-MIA-injection ($p > 0.05$).

**IL-1β and TNF-α level in synovium**

The concentrations of IL-1β and TNF-α are shown in Figure 4. In the control group, the concentrations of IL-1β and TNF-α remained at a low level during the observation period. Compared to the controls, IL-1β and TNF-α levels in the MIA group were significantly increased on Day 1 and reached a maximum level on Day 3 ($p < 0.05$), whereas no difference was observed between the two groups on Days 7, 14, 21 and 42 ($p > 0.05$).

**Pharmacological assessment**

The effects of celecoxib and gabapentin on mechanical hyperalgesia at different time points are shown in Figure 5. A significant reduction in PWT was only found on Day 3 ($p < 0.05$) in the celecoxib group, whereas there was a lack of inhibition on Days 21 and 35 ($p > 0.05$). However, administration of gabapentin significantly reduced mechanical hyperalgesia on post-injection Days 3, 21 and 35 ($p < 0.05$).

Figure 2. Degeneration of articular cartilage was assessed by modified Mankin’s scores, which differed significantly between control rats and those treated with monosodium iodoacetate (MIA) at all-time points (*$p < 0.05$ vs. controls).

Figure 3. Behavioral tests for mechanical hyperalgesia in rats treated with monosodium iodoacetate (MIA) or saline (control) were evaluated on Days 1, 3, 7, 14, 21, 28, 35 and 42 ($n = 10$ on each day for each group). A biphasic pattern of change in paw withdrawal threshold was observed in the MIA group. Compared to the control group, a significant reduction in paw withdrawal threshold (PWT) was noted in the MIA group on Days 1, 3, 21, 28, 35 and 42 (*$p < 0.05$ vs. controls).

Figure 4. Concentrations of (A) interleukin (IL)-1β and (B) tumor necrosis factor (TNF)-α in the synovium of rats treated with monosodium iodoacetate (MIA) or saline (control) were measured by enzyme-linked immunosorbent assay on Days 1, 3, 7, 14, 21 and 42 ($n = 3$ on each day for each group). Compared to controls, IL-1β and TNF-α levels were significantly increased on Day 1 and reached a maximum level on Day 3 ($p < 0.05$) in the MIA group, whereas no difference was observed between the two groups on Days 7, 14, 21 and 42 ($p > 0.05$) (*$p < 0.05$ vs. controls).
Discussion

LFJ is a true synovial joint with articular cartilage, synovial membrane, and fibrous joint capsule.\textsuperscript{12} According to the cadaveric study of Tischer et al,\textsuperscript{13} the pathological characteristics of human LFJ OA are progressive degeneration of articular cartilage and subsequent changes in subchondral bone. There have been only two studies that have reported experimental models of LFJ OA. A surgical model in sheep was introduced by Moore et al in 1999.\textsuperscript{7} OA-like histopathological changes in the LFJs of sheep could be induced by annular rim incision of the discs. The surgical model has the advantage that the cause of OA is due to the loading change followed by disc incision and subsequent degeneration, which is similar to the etiology of LFJ OA in humans.\textsuperscript{14} However, the usefulness of surgical models is limited by the extended time frame (18 months) required for the classic features of OA to develop. In addition, it is hard to mimic completely the natural progress of LFJ OA in humans, owing to the difference in anatomy and loading between humans and animals. Besides the surgical model, Yeh et al\textsuperscript{9} have reported a collagenase-induced LFJ OA model. However, measurement of facet joint pain or relative referred pain was not mentioned or included in either of the above models. The model in our study, which was similar to the collagenase-induced one, was time-saving and reproducible. Histopathological changes that closely mimicked human OA lesions were observed within 6 weeks after intra-articular injection of MIA. The pattern of facet joint degeneration was time-dependent and comprised the features of LFJ OA-like changes at different stages. In addition, facet-mediated mechanical hyperalgesia was observed in our MIA-induced model.

According to Tachihara et al,\textsuperscript{8} facet-mediated mechanical hyperalgesia also could be found in a rat model of LFJ inflammation. By administering a low volume of complete Freund’s adjuvant (CFA) into a single facet joint, inflammation, cartilage degeneration, and concomitant pain-related behavioral changes were induced. Tachihara et al\textsuperscript{8} have suggested that facet joint inflammation, and not degeneration of the joint, might have been a cause of pain. However, there are two main differences between their CFA-induced model and
our model. First, the degree of cartilage degeneration in the former was mild or moderate and was constant during the observation period, whereas the cartilage degeneration in our MIA-induced OA model was progressive and severe, and subsequent changes in subchondral bone could be observed more than 21 days after injection. Second, a reduction in mechanical threshold was noted on Days 3, 5 and 7 in the CFA-induced model, whereas a biphasic pattern of mechanical hyperalgesia was seen within 6 weeks in our MIA-induced model.

In the present study, changes in IL-1β and TNF-α levels were closely associated with the first phase of facet-mediated mechanical hyperalgesia (1–7 days). The synovium is richly innervated by free nerve endings and inflammatory cytokines can spread to the epidural space; therefore, it can be assumed that inflammation played a role in the first phase of the facet-mediated mechanical hyperalgesia in our MIA-induced model. The inhibitory effect of celecoxib on mechanical hyperalgesia at Day 3 also suggested an early component of inflammatory pain in our model. However, the facet joint inflammation induced by MIA was transient and largely resolved by Day 7 in our study. Therefore, it would not be expected to mediate the second phase of facet-mediated mechanical hyperalgesia on Days 21, 28, 35 and 42. During the late observation period, severe degenerative changes in the facet joint were found, including exposure of subchondral bone, bone marrow fibrosis, and marginal osteophyte formation. According to Mach et al, subchondral bone and bone marrow were innervated with endings of small-diameter axons and could potentially be a source of pain. Niva et al have indicated that exposure of subchondral bone, bone marrow lesions, and osteoclast activity might be the major causes of joint pain in OA. Therefore, we assume that the second phase of facet-mediated mechanical hyperalgesia in the MIA-induced OA model might have been partly due to these severe degenerative changes. In addition, we found that gabapentin was able to reverse mechanical hyperalgesia on Days 21 and 35, whereas celecoxib lost its inhibitory effect on Days 21 and 35. This suggests that the second phase of facet-mediated mechanical hyperalgesia can be regarded as neuropathic pain relative to behavioral changes. However, further studies are needed to elucidate the exact mechanism.

In summary, we describe a reproducible MIA-induced rat model of LFJ OA that mimics the pathological features of human OA. In addition, obvious facet-mediated mechanical hyperalgesia was found during the whole observation period. Although this model could only be regarded as a preliminary animal model of facet joint OA pain, it might provide a useful tool for further study to ascertain the complex mechanism involved.

References


