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Mono-Iodoacetate-Induced Histologic Changes in Subchondral Bone and Articular Cartilage of Rat Femorotibial Joints: An Animal Model of Osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is a degenerative joint disease characterized by joint pain and a progressive loss of articular cartilage. Studies to elucidate the pathophysiology of OA have been hampered by the lack of a rapid, reproducible animal model that mimics both the histopathology and symptoms associated with the disease. Injection of mono-iodoacetate (MIA), an inhibitor of glycolysis, into the femorotibial joint of rodents promotes loss of articular cartilage similar to that noted in human OA. Here, we describe the histopathology in the subchondral bone and cartilage of rat (Wistar) knee joints treated with a single intra articular injection of MIA (1 mg) and sacrificed at 1, 3, 5, 7, 14, 28, and 56 days postinjection. Histologically, the early time points (days 1–7) were characterized by areas of chondrocyte degeneration/necrosis sometimes involving the entire thickness of the articular cartilage in the tibial plateaus and femoral condyles. Changes to the subchondral bone, as evidenced by increased numbers of osteoclasts and osteoblasts, were noted at by day 7. By 28 days, there was focal fragmentation and collapse of bony trabeculae with fibrosis and increased osteoclastic activity. By 56 days there were large areas of bone remodeling evidenced by osteoclastic bone resorption and newly formed trabeculae with loss of marrow hematopoietic cells. Subchondral cysts and subchondral sclerosis were present in some rats. In conclusion, intra-articular injection of MIA induces loss of articular cartilage with progression of subchondral bone lesions that mimic those of OA. This model offers a rapid and minimally invasive method to reproduce OA-like lesions in a rodent species.

Keywords. Monoiodoacetate; osteoarthritis; femorotibial joint; articular cartilage; subchondral bone; osteoclasts; animal model.

INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease characterized by joint pain and a progressive loss of articular cartilage (17). About 80% to 90% of individuals of both sexes have evidence of OA by the time they reach age 65 (10). The triggering events and exact pathologic mechanism that result in cartilage loss and degradation are not completely understood. It has been suggested that biochemical alterations occur within the articular cartilage resulting in imbalances between synthetic and degradative pathways (8). Concurrent with these biochemical alterations are changes in the joint cartilage and bone. Changes that occur within the cartilage include fibrillation and splitting of the noncalcified cartilage with subsequent thinning of the cartilage layer. The underlying bone is characterized by increased osteoclast and osteoblast activity, resulting in altered bone contour and formation of subchondral cysts (20).

In order to study the pathogenesis of this disease and to evaluate the effectiveness of novel therapeutic agents, it is desirable to have an animal model that consistently reproduces the joint pathology and pain associated with OA. The disease develops naturally in several strains of mice or can be induced in other species by surgically disrupting joint structures such as the cruciate ligaments or menisci (1). The usefulness of these models is limited by the extended time frame required for the classic features of OA to develop. Therefore a model that rapidly reproduces the clinical and pathologic features of OA is very desirable. Cartilage degeneration can also be induced by administration of quinolone antibiotics or by intra-articular injection of iodoacetates (2). Mono-iodoacetate (MIA) is an inhibitor of glyceraldehyde-3-phosphate dehydrogenase activity, and therefore an inhibitor of glycolysis shown to induce chondrocyte death in vitro (4). Intra-articular injection of MIA induces chondrocyte death in the articular cartilage of rodent and nonrodent species (6).

When used in rodents, the model reproduces cartilage lesions with loss of proteoglycan matrix and functional joint impairment similar to human OA (19). In cartilage, lesions are characterized by chondrocyte necrosis, cell cloning (chondrones), fibrillation, loss of stainable proteoglycan matrix, and erosion with exposure of subchondral bone. Reported bone lesions include remodeling and sclerosis of subchondral bone with osteophyte formation (19, 9). Although the use of this model and the associated cartilage changes have been reported, the early progression of lesions from chondrocyte death to subchondral bone pathology has not been previously described. Unlike articular cartilage, subchondral bone is richly innervated and could potentially be a source of pain (7). Since pain is a major cause of incapacity due to OA, it is important to understand how histologic changes correlate with the onset of pain or joint impairment. In this report, we describe the chronologic progression of histologic lesions in the articular cartilage and subchondral bone of rat femorotibial joints at different time points up to 56 days post-MIA injection.

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MATERIALS AND METHODS

Induction of Osteoarthritis: The procedures used in this study were in agreement with the guidelines of the Pfizer Institutional Animal Care and Use Committee (IACUC). Animals were provided with living conditions, food, and housing consistent with the approved animal care operating procedures. Male Wistar rats (175–200 g; Charles River, Wilmington, MA) were allowed to acclimate to the facility for 7 days. Animals were fed standard rat chow with water available ad libitum. In order to minimize any potential discomfort, animals were housed in solid bottom cages (2 per cage) as opposed to wire bottom cages. In addition, the animals were closely monitored throughout the duration of the protocol (56 days) by members of the Laboratory Animal Resources Unit. It should be noted that the animals retained full mobility and continued to grow normally. For induction of MIA-induced arthritis, rats were anesthetized with isoflurane (Abbott Laboratories, North Chicago, IL, USA) and given single intra-articular injection of 1 mg of monosodium iodoacetate (MIA; Sigma, St. Louis, MO, USA; cat #I2512) through the infrapatellar ligament of the right knee. MIA was dissolved in physiologic saline and administered in a volume of 50 μl using a 27-gauge, 0.5-inch needle. The left contralateral control knee was injected with 50 μl of physiologic saline. The amount of MIA injected into the joint was determined from a dose-response study in which the maximal degree of joint discomfort was noted using a concentration of 1 mg/joint (3).

Histology: Groups of 4 animals were euthanized by CO_2 asphyxiation at 1, 3, 5, 7, 14, 28, and 56 days post-MIA injection. Soft tissue was removed from the medial and lateral joint capsule from MIA and contralateral saline treated femorotibial joints. Tissue samples were prepared for light microscopy using standard procedures. Briefly, samples were fixed in 10% phosphate-buffered formalin and subsequently decalcified in 5% formic acid for 72 hours. Frontal sections of femorotibial joints were routinely processed to HE slides and examined under the light microscope.

RESULTS

At day 1 post-injection, there were extensive areas of chondrocyte degeneration that frequently involved the entire thickness of the articular cartilage. Chondrocytes were shrunken with a hypereosinophilic cytoplasm and fragmented pyknotic nuclei (Figure 1). Chondrocyte degeneration was present in the femoral condyles and as well as the tibial plateaus, however, the medial tibial plateau was most consistently affected. The synovial membrane was expanded by fibrin and proteinaceous edema fluid mixed with mild to moderate infiltrates of lymphocytes, macrophages and plasma cells.

By days 5 and 7 the inflammatory response in the synovium had subsided and there was moderate collapse of the cartilaginous matrix with marked loss of chondrocyte cellular detail (Figure 2). Involvement of subchondral bone was not seen until day 7 and consisted of increased numbers of osteoclasts along the junction between damaged necrotic cartilage and subchondral bone. The cells were multinucleated and some were partially embedded within the osteoid matrix. Some adjacent trabeculae were lined by single to several rows of large, reactive osteoblasts (Figure 3). These cells were cuboidal to tall columnar with basally located nuclei, abundant basophilic cytoplasm, and prominent Golgi apparatus.

At 14 days, focally extensive areas of the subchondral bone marrow were replaced by loosely arranged spindle cells contained within a fine stroma. These areas of bone marrow replacement were present underneath damaged cartilage and were sharply demarcated from normal trabecular bone and marrow elements (Figure 4). Variably sized clusters of chondrocytes were present at the zone of demarcation between viable and necrotic cartilage (chondrones).

At 28 days, there was multifocal collapse and fragmentation of bony trabeculae. Fragmented bone was surrounded by numerous osteoclasts and was contained within focally

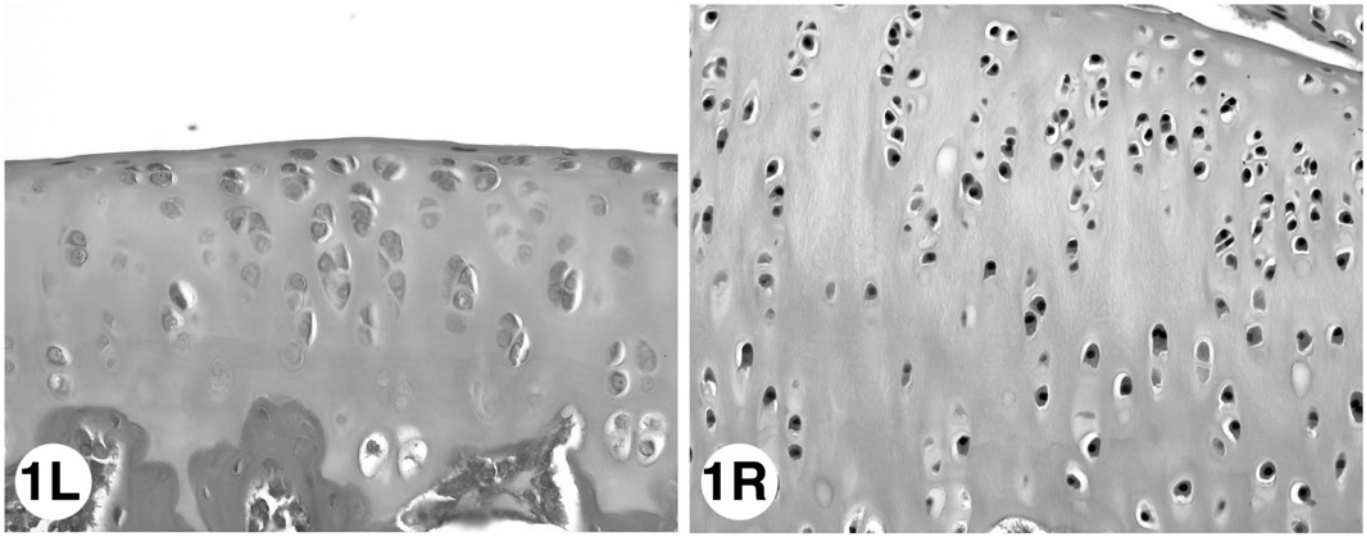

FIGURE 1.—Articular cartilage from Wistar rat femoro-tibial joint, 1 day post-MIA injection. Right panel, note extensive chondrocyte degeneration in tibial plateau. Left, cartilage from a joint injected with saline. H & E, ×400.

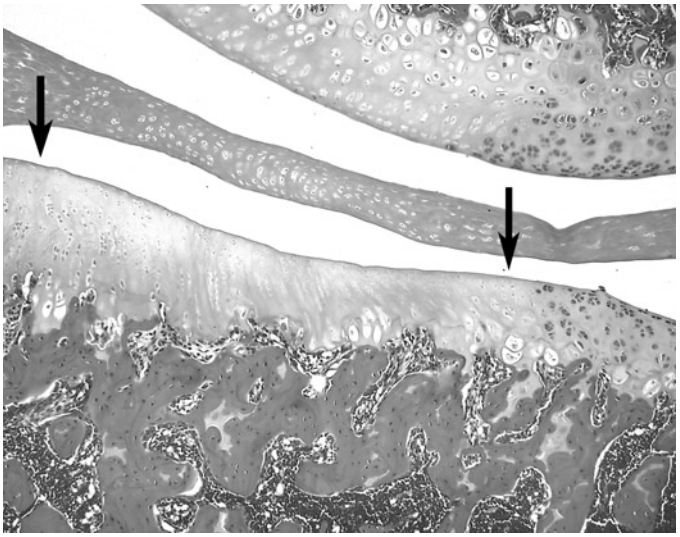

FIGURE 2.—Femoro-tibial joint, Wistar rat, 5 days post-MIA injection. Note collapse of necrotic articular cartilage. There is marked loss of chondrocyte cellular detail. H & E, ×100.

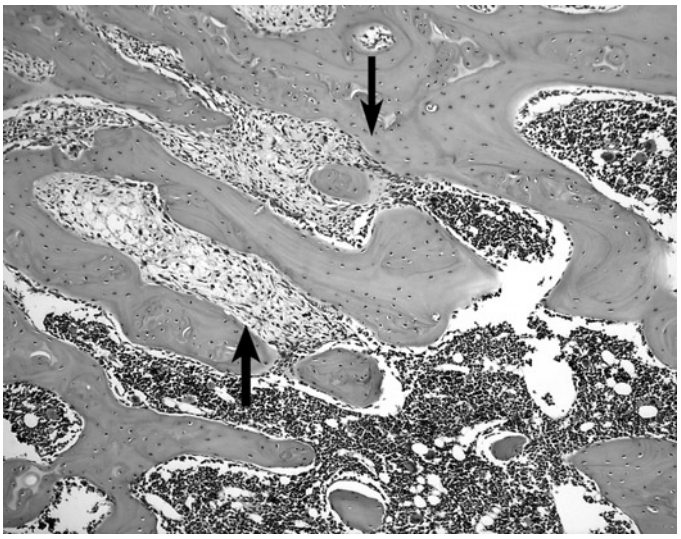

FIGURE 4.—Femoral condyle, Wistar rat, 14 days post-MIA injection. Bone marrow elements are replaced by loosely arranged spindle cells in a fine fibrous stroma (arrows) H & E, ×100.

extensive areas of fibrosis that replaced adjacent bone trabeculae and marrow elements (Figures 5 and 6). Scant cellular debris was also present in these areas. Gross lesions were first visible at 28 days and consisted of a well-demarcated area of cartilage erosion, which was most commonly present in the medial tibial plateau (Figure 7). Gross lesions were not observed at 1, 3, 5, or 7 days.

At 56 days, newly laid trabecular bone was sometimes present within areas of bone resorption and active osteoclastic activity (bone remodeling). Bone marrow spaces were mostly devoid of hematopoietic elements and contained scant

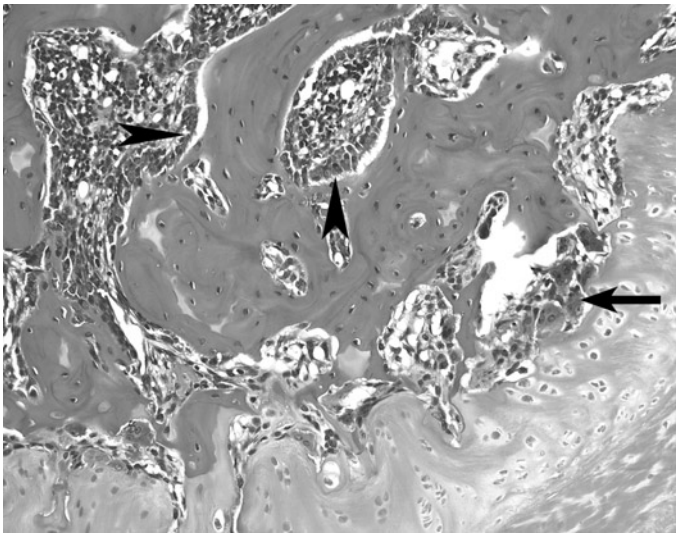

FIGURE 3.—Femoro-tibial joint, Wistar rat, 7 days post-MIA injection. Increased osteoclastic activity in subchondral bone in tibial plateau (arrow). Some bony trabeculae are lined by prominent rows of osteoblasts (arrowheads). H & E, ×200.

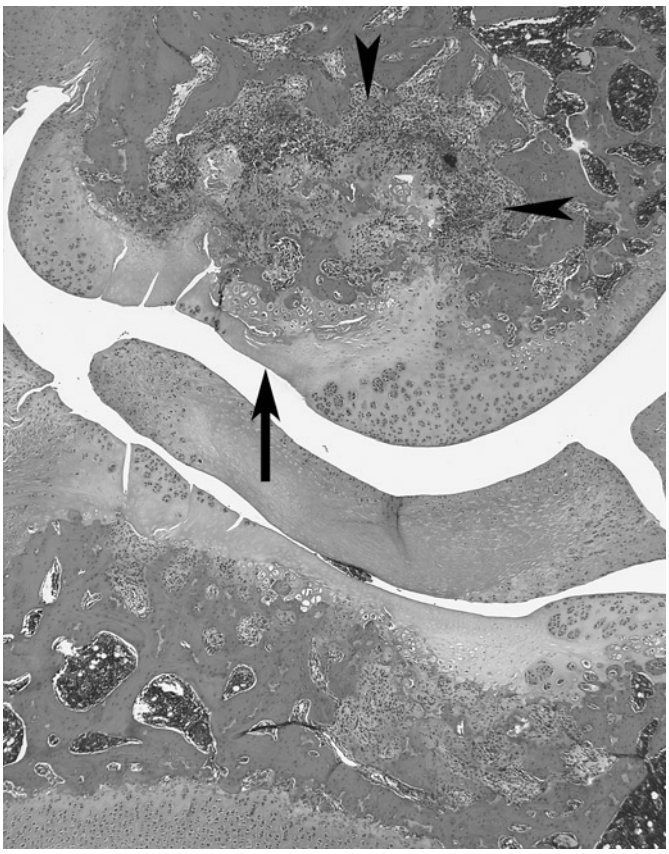

FIGURE 5.—Femoro-tibial joint, Wistar rat 28 days post-MIA injection. Note focally extensive area of cartilage loss and degeneration in the femoral condyle (arrows). There is subchondral bone collapse and fragmentation with replacement by fibrous tissue. H & E, ×40.

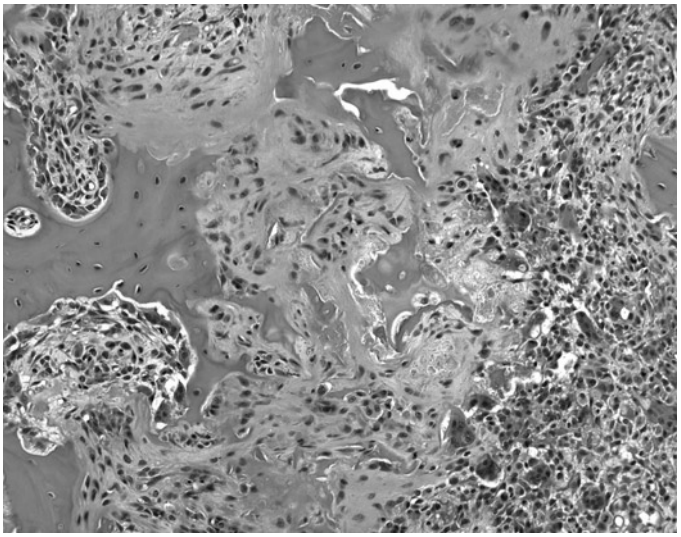

FIGURE 6.—Higher magnification of subchondral bone from Figure 6. Note fragmented trabeculae surrounded by osteoclasts and fibrous tissue. H & E, ×200.

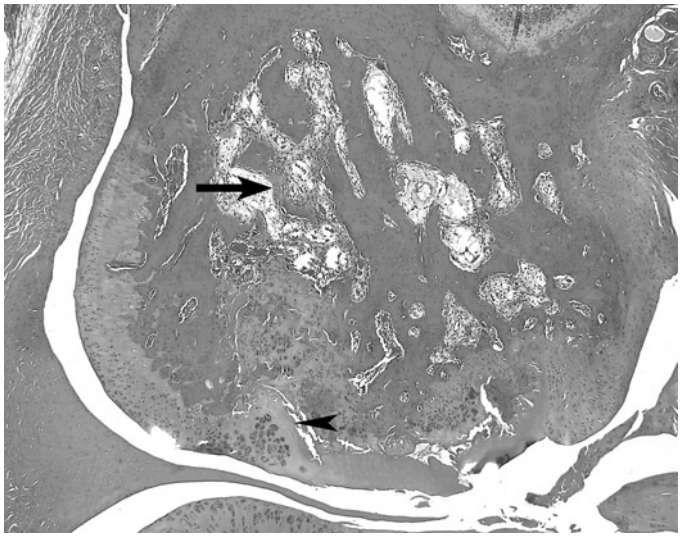

FIGURE 8.—Femoral condyle, Wistar rat, 56 days post-MIA. Note loss of hematopoietic elements in marrow elements associated with bone resorption and new trabecular bone formation (arrow). Associated articular cartilage is fragmented and necrotic. Note areas of chondrocyte proliferation that invaginate into the subjacent subchondral bone (Figure 8). H & E, ×40.

proteinaceous debris or loose mesenchymal tissue. Associated articular cartilage was usually fragmented and necrotic, but there were areas of chondrocyte proliferation that invaginated into the subjacent subchondral bone (Figure 8). Cystic structures demarcated by fibrous tissue and containing scant necrotic debris were sometimes present (Figure 9). Areas of subchondral sclerosis were occasionally seen.

DISCUSSION

Although the use of intra-articular MIA in animal models of osteoarthritis has been previously reported, the present study outlines the time-dependent occurrence and progression of histologic lesions with major emphasis on subchondral bone, a potential source of OA associated pain. Differences in hind paw weight-bearing potential as a measure of joint pain have been evaluated in this animal model. A concentration dependent increase in joint pain was noted after intra-articular injection of MIA, that was reduced by the administration of nonsteroidal anti-inflammatory agents (3). The pathogenesis and source of pain in OA are uncertain. Although articular cartilage is primarily affected by the disease, it does not contain nerve fibers. Additional anatomic structures that have been proposed as potential sources of pain include the synovial capsule/membrane, menisci and subchondral bone (5, 21, 22). Since subchondral bone is richly

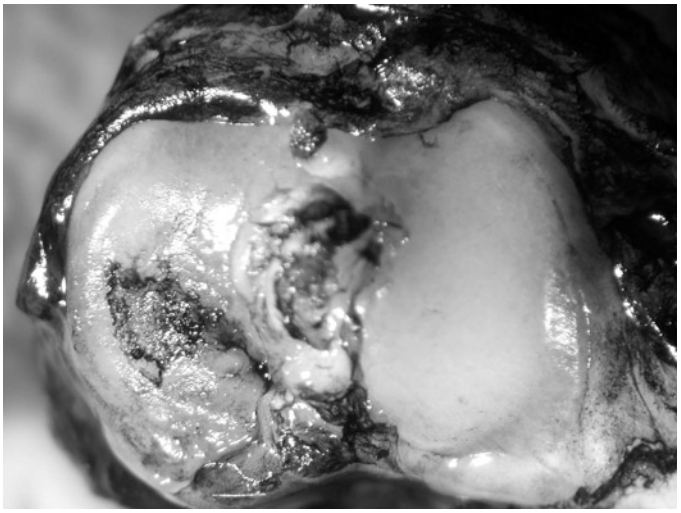

FIGURE 7.—Macroscopic changes in tibial articular cartilage, Wistar rat 28 days post-MIA injection. Focally extensive area of cartilage loss and erosion in the medial tibial plateau.

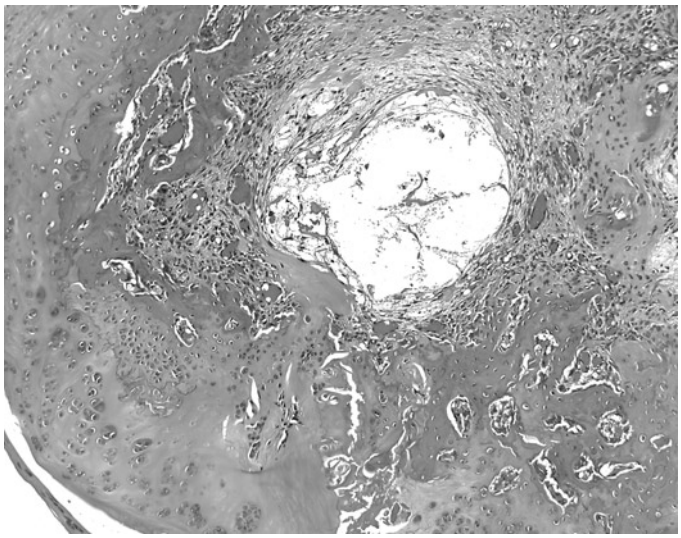

FIGURE 9.—Femoral condyle, Wistar rat, 56 days post-MIA. There is a moderately well-defined subchondral cystic structure demarcated by fibrous tissue. H & E, ×100.

innervated, it is important to understand how the occurrence and progression of bone lesions relate to the onset of joint pain.

In our study, evidence of subchondral bone involvement was apparent as early as 7 days post-MIA. These changes consisted of increased osteoclastic and osteoblastic activity in the trabecular bone immediately subjacent to the areas of cartilage loss and degeneration. The changes are consistent with the initiation of bone remodeling and may have been induced by increased load in the subchondral bone due to the advanced loss of cartilage. There was a close anatomic relationship between the damaged cartilage and changes in the subchondral bone. By day 7, cartilage damage was characterized by complete loss of cellular detail of chondrocytes, with thinning and collapse of the cartilaginous matrix. Our results are consistent with those reported by others in that bone lesions consistent with bone resorption, begin to develop within 7 days post-MIA (13).

The mechanisms that mediate bone pain are complex and may involve release of inflammatory cytokines, nerve damage, and ongoing osteoclastic activity (18). Increased osteoclastic activity was reported in a rat model of bone cancer pain in which mammary cancer cells were directly injected into the tibia. Progression of the tumor was associated with increased numbers of osteoclasts in the adjacent bone and reduced weight bearing on the affected leg (15). In a murine model, bone cancer pain was partially blocked by elimination of osteoclastic activity, even in cases of advanced bone cancer (11, 14). It is therefore possible that initiation of osteoclastic activity as part of bone remodeling seen in this model and in human OA mediates, at least in part the associated pain.

By 14 days, the bone marrow spaces immediately adjacent to the damaged cartilage were filled with loose spindle cells consistent with fibrosis. Fibrosis of subchondral bone marrow was also reported in human metacarpal and metatarsal heads resected surgically from patients with advanced rheumatoid arthritis, features that were judged to be consistent with bone repair. As in this study, areas of bone marrow fibrosis correlated with the presence of necrotic cartilage (23).

At day 28 there was focal compression and fragmentation of subchondral bone with replacement of bone and marrow elements by mature, dense fibrous tissue. At 56 days, there were areas of bone resorption with concurrent new trabecular bone formation. Areas of bone lysis as well as adjacent bone marrow spaces were devoid of hematopoietic elements and occupied only by few, fine and widely spaced spindle cells containing scant finely granular proteinaceous material and necrotic bone fragments. These lesions are consistent with accumulation of fluid within marrow spaces and areas of bone lysis. Humans with knee pain and osteoarthritis were found to have magnetic resonance imaging (MRI) lesions in subchondral bone consistent with of bone marrow edema, suggesting that there is a correlation between this bone marrow lesion and pain in knee osteoarthritis (7). In addition, the development of subchondral cysts is a characteristic feature of human OA (20) and resemble those seen in our study at 56 days postinjection.

Histology was the only endpoint evaluated in this study, however, additional tools such as radiology or micro-CT (computerized tomography) technology could be used to monitor the progression of subchondral bone changes in vivo, especially in efficacy studies. Although biochemical profiles indicative of bone turnover such as bone specific alkaline phosphatase (16) or osteocalcin (12) were not measured in blood or urine, histologic findings suggest that this model would be suitable for biomarker development. Measurement of serum biomarkers or bone labeling techniques could be more sensitive than routine histology in the assessment of bone turnover in osteoarthritis. Since those techniques were not applied in the current study, we cannot exclude the possibility that bone changes undetectable by histology could occur earlier than at 7 days.

In conclusion, intra-articular injection of MIA induces loss of articular cartilage with subchondral bone lesions that mimic those of human OA. It is a rapid and minimally invasive method to induce OA-like lesions in a rodent species. The model demonstrates a clear interrelationship between cartilage damage and bone changes and could be used to study the role of subchondral bone in the progression of OA. With the development of methodology to monitor joint discomfort in laboratory animals, this model could be used to correlate the onset and progression of pain with specific joint lesions.

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