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In Vivo Microfocal Computed Tomography and Micro–Magnetic Resonance Imaging Evaluation of Antiresorptive and Antiinflammatory Drugs as Preventive Treatments of Osteoarthritis in the Rat

Michael D. Jones,[1] Charles W. Tran,[1] Guang Li,[1] Walter P. Maksymowych,[1] Ronald F. Zernicke,[2] and Michael R. Doschak[1]

Objective. **To determine whether treatment with an antiresorptive drug in combination with an antiinflammatory drug reduces periarticular bone and soft tissue adaptations associated with the progression of posttraumatic secondary osteoarthritis (OA).**

Methods. **We used in vivo microfocal computed tomography (micro-CT) to map bony adaptations and in vivo micro–magnetic resonance imaging (micro-MRI) to examine joint inflammation in a rat model of surgically induced OA secondary to knee triad injury. We examined the arthroprotective effects of the bisphosphonates alendronate and risedronate and the nonsteroidal antiinflammatory drug (NSAID) meloxicam.**

Results. **Micro-CT revealed reduced levels of periarticular trabecular bone loss in animals with knee triad injury treated with the bisphosphonate drugs alendronate or risedronate, or the NSAID meloxicam, compared with untreated animals. Alendronate treatment reduced bony osteophyte development. While risedronate as a monotherapy did not positively impact osteophytogenesis, combination therapy with risedronate and meloxicam reduced osteophyte severity somewhat. Micro-MRI revealed an increased, diffuse water signal in the epiphyses of untreated rats with knee triad injury 8 weeks after surgery, suggestive of a bone marrow lesion–like stimulus. In contrast, meloxicam-treated rats showed a significant reduction in fluid signal compared with both bisphosphonate-treated groups 8 weeks after surgery. Histologic analysis qualitatively confirmed the chondroprotective effect of both bisphosphonate treatments, showing fewer degradative changes compared with untreated rats with knee triad injury.**

Conclusion. **Our findings indicate that select combinations of bisphosphonate and NSAID drug therapy in the early stages of secondary OA preserve trabecular bone mass and reduce the impact of osteophytic bony adaptations and bone marrow lesion–like stimulus. Bisphosphonate and NSAID therapy may be an effective disease-modifying drug regimen if administered early after the initial injury.**

Traumatic injury to the knee joint (such as ligament disruption or meniscal damage) predisposes to secondary osteoarthritis (OA), a debilitating disease of the joints characterized by the focal deterioration and eventual full-thickness loss of articular cartilage and by periarticular bone and soft tissue adaptations. Radiographic evidence of OA includes narrowing of the joint space after cartilage damage, with accompanying changes in periarticular trabecular bone and/or osteophyte formation at the joint margins and ligament entheses. Accordingly, periarticular bony adaptations may be useful as progressive prognostic indicators (and as potential therapeutic drug targets) of OA, particularly during the early stages of OA pathogenesis (1,2).

Osteophytes arise on bone at the synovial joint margins, from activated cells of the periosteum, initially appearing as cartilaginous growths that later calcify via

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[1]Michael D. Jones, MSc, Charles W. Tran, BSc, Guang Li, MSc, Walter P. Maksymowych, MD, FRCPC, Michael R. Doschak, PhD, MSc: University of Alberta, Edmonton, Alberta, Canada; [2]Ronald F. Zernicke, PhD, DSc: University of Michigan, Ann Arbor.

Address correspondence and reprint requests to Michael R. Doschak, PhD, MSc, Pharmacy and Pharmaceutical Sciences, University of Alberta, 2121 Dentistry/Pharmacy Centre, Edmonton, Alberta T6G 2N8, Canada. E-mail: mdoschak@ualberta.ca.

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endochondral ossification (3). Mediators of inflammation secreted by the synovium, such as interleukin-1, tumor necrosis factor α, and transforming growth factor β, are believed to be involved in osteophytogenesis (4,5). Osteophytes are known to cause joint stiffness by limiting the movement of the joint (6) and leading to fixed bony deformity, and thus are permanent bony changes that increase the severity of OA, and are strong predictors of OA-related pain (7). Therefore, the reduction of osteophyte incidence using drugs known to influence bone mass (such as the bisphosphonates), and/or drugs capable of blocking the potential costimulation of osteophytogenesis by mediators of inflammation (such as nonsteroidal antiinflammatory drugs [NSAIDs]), remains a viable disease-modifying goal of OA therapy.

Our study focused on the reported ability of alendronate to block osteophytosis (8), a process that the closely related bisphosphonate drug risedronate has not been shown to influence (9). The aim of our study was to determine whether the use of bisphosphonate drugs at an early stage of OA pathogenesis reduced periarticular bony adaptations (such as osteophyte severity) and/or cartilage degradation in OA. We also examined whether the addition of an NSAID to that regimen would further reduce osteophytic OA joint adaptations. The NSAID meloxicam is a selective cyclooxygenase 2 (COX-2) inhibitor (10), with known antiinflammatory and antiangiogenic effects (11–13). NSAIDs are prescribed primarily for the management of OA pain (14,15); however, they have also been found to have positive effects on cartilage health in OA by increasing proteoglycan and hyaluronan synthesis (16). Thus, we hypothesized that the addition of an NSAID to the bisphosphonate drug regimen may further mediate the reduction of the inflammatory stimulus and prove beneficial in preventing or slowing osteophytes and cartilage degradation. Our study further tested the utility of 2 different multimodal noninvasive imaging technologies (high-resolution in vivo microfocal computed tomography [micro-CT] and in vivo micro–magnetic resonance imaging [micro-MRI]), with subsequent histologic validation, to assess mineralized and soft tissue adaptations indicative of OA.

MATERIALS AND METHODS

Surgically induced OA in an animal model. Fifty-eight 6-week-old (~275-gm) female Sprague-Dawley rats were obtained from the University of Alberta Biosciences Animal Services. The protocol pertaining to all procedures and aspects of the study was approved by the University of Alberta animal care and ethics committee. OA pathogenesis was surgically induced in 46 of the rats, using the surgical knee triad injury model of knee OA, performed as previously described (17). Only the right knee (stifle) joint was operated upon. All rats received a single dose of an opioid analgesic (buprenorphine) on the first day after surgery only, since rats resumed active caged activity with no indication of morbidity. Briefly, a medial capsulotomy was performed, and knee triad injury was induced in rats by transection of the medial collateral ligament (MCL) at its midpoint, removal of the medial meniscus after severing the anchoring horns, and midsubstance transection of the anterior cruciate ligament (ACL). Eight rats underwent sham surgery in which the joint was surgically exposed, and the MCL, medial meniscus, and ACL were manipulated but left intact. Sterile saline was used to flush joint surfaces, and the capsule and skin were sutured using 5-0 Vicryl. Surgery was performed using 1.5% isoflurane/L oxygen inhalation anesthesia. All animals were killed 8 weeks after surgery, and the femur and tibia were then isolated and fixed in neutral buffered formalin (pH 7.0).

Experimental design. Our investigation was conducted in 2 stages, using age-matched cohorts of rats. The initial phase focused on the comparison of risedronate and alendronate as disease-modifying OA drugs (DMOADs). Six-week-old (~275-gm) female Sprague-Dawley rats were randomly allocated into the following 4 groups: rats with knee triad injury treated with alendronate (n = 8), rats with knee triad injury treated with risedronate (n = 8), untreated rats with knee triad injury (n = 6), and untreated sham-operated rats (n = 8).

The second stage focused on the assessment of the potential of meloxicam as a DMOAD, either as a monotherapy or in combination with a bisphosphonate drug. For all in vivo micro-MRI measurements, a random subset of 4 rats per group was imaged due to the expense of operating the 9.4T system. For this stage of the study, a second cohort of 6-week old (~275-gm) female Sprague-Dawley rats was obtained from the same supplier (University of Alberta Biosciences Animal Services) and randomly allocated into the following 5 groups: rats with knee triad injury treated with meloxicam (n = 8), rats with knee triad injury treated with meloxicam and risedronate (n = 8), rats with knee triad injury treated with risedronate (n = 4), untreated rats with knee triad injury (n = 4), and untreated uninjured rats (n = 4).

Drug dosage. All treatments lasted for 8 weeks, from the day after surgery until the experimental end point (when rats were killed). Eight rats with knee triad injury received 0.12 mg/kg body mass of alendronate twice weekly by subcutaneous injection. This concentration corresponded to the "high" dose established in previous studies using a rat model of OA (8). Risedronate 0.06 mg/kg body mass was administered twice weekly by subcutaneous injection to 12 rats with knee triad injury and to 8 rats with knee triad injury that were also treated with meloxicam. This corresponded to an effective dose for preventing bone loss that was established in previous studies (9). The dosage of risedronate was half that of alendronate, due to the increased potency of risedronate, and corresponded with the relative dosing currently used in bisphosphonate treatment of osteoporosis in humans.

Meloxicam was administered to rats by daily oral gavage at a dose of 3 mg/kg as previously described (18), using an 18-gauge, 2-inch curved animal feeding needle (Harvard Apparatus). The meloxicam was suspended in a 60:40 solution

Table 1. Bone volume percentage, osteophyte severity scores, and modified Mankin scores 8 weeks after knee triad injury surgery in rats*

Treatment group	Bone volume percentage	Osteophyte severity score†	Modified Mankin score‡
Sham-operated rats (n = 8)	46.45 ± 7.5	0.10 ± 0.01	0.25 ± 0.00§
Untreated rats with knee triad injury (n = 6)	37.41 ± 8.4	2.00 ± 0.16¶	5.01 ± 0.70
Alendronate-treated rats with knee triad injury (n = 8)	49.51 ± 9.2§	1.38 ± 0.38¶	3.09 ± 0.36
Risedronate-treated rats with knee triad injury (n = 8)	42.06 ± 4.4	1.94 ± 0.29¶	3.31 ± 0.31
Meloxicam-treated rats with knee triad injury (n = 8)	64.35 ± 4.4#	1.88 ± 0.26¶	4.83 ± 0.75
Combination-treated rats with knee triad injury (n = 8)	56.17 ± 7.1§	1.69 ± 0.27¶	3.00 ± 0.63
Untreated uninjured rats (n = 8)	55.52 ± 4.2§	NA	NA

* Values are the mean ± SEM. Rats with knee triad injury had surgically induced osteoarthritis. Rats in the combination treatment group were treated with a combination of risedronate and meloxicam. NA = not applicable.
† Higher scores (maximum 3.0) indicate greater osteophyte severity.
‡ Higher scores (maximum 13.0) indicate greater cartilage damage.
§ $P < 0.05$ versus untreated rats with knee triad injury.
¶ $P < 0.05$ versus sham-operated rats.
# $P < 0.05$ versus combination-treated rats with knee triad injury, risedronate-treated rats with knee triad injury, untreated rats with knee triad injury, and untreated uninjured rats.

of propylene glycol and distilled water at a concentration of 1 mg/ml. For meloxicam administration, food was withheld from rats for 2 hours before and after drug dosing. Vehicle was injected subcutaneously twice weekly in untreated rats with knee triad injury and sham-operated rats. For combination therapy, the bisphosphonate was injected first subcutaneously, and then food was withheld from rats for 2 hours before and after meloxicam dosing as described above. To confirm the plasma levels of meloxicam, blood samples were obtained from rats at T_{max} (time to maximum plasma concentration) after an oral dose of meloxicam and sampled after in vivo micro-CT imaging while rats were under anesthesia. Plasma levels of meloxicam were determined using reverse-phase high-performance liquid chromatography (HPLC) as previously described (18). HPLC conditions consisted of a buffered mobile phase of 50 m*M* sodium phosphate, methanol, and acetonitrile in a 5:4:1 ratio (volume/volume). Flow rate was 1.0 ml/minute with a C-18 solid-phase column and an ultraviolet detection wavelength of 364 nm.

In vivo micro-CT. In vivo micro-CT was performed at 3 time points: 1 day prior to surgery, 4 weeks after surgery, and when rats were killed 8 weeks after surgery. Isoflurane (Halocarbon) was used as the anesthetic for the duration of the procedure (~35 minutes). Rats were placed supine in the bed of a Skyscan 1076 in vivo X-ray microtomograph. The right hind limb was extended and secured to the gantry bed using masking tape to limit movement. Radiographs encompassed the entire stifle joint in a single field of view. Two-dimensional (2-D) projections were obtained using an x-ray source setting of 70 kV and 139 μA, with beam filtration through a 1.0-mm aluminum filter. Data were collected every 0.5° rotation step through 180°. The scanning width was 35 mm, and the height was 17 mm. Reconstruction was performed using a modified Feldkamp back-projection algorithm with a cross-section to image of 0.0000–0.0602. The resulting slices were composed of isotropic voxels with a resolution of 17.46 μm^3. Bone morphometry was determined using Skyscan CT-Analyser Software. A 1.53-mm^3 sphere was centered in the epiphyseal trabecular bone of the medial condyle of the distal femur and sampled to assess the bone volume percentage. The sphere defined a volume of interest that included only trabecular bone, and a single observer (MDJ) performed the measurements to improve consistency in sphere placement. Micro-CT images were binarized with a grayscale threshold of 60/255.

To assess osteophyte severity, 2 independent observers (MDJ and GL) who were blinded with regard to treatment group visually scored 3-D micro-CT models of the femur and tibia from each rat. Osteophyte severity was graded on a 4-point scale, where 0 = no observed osteophytes, 1 = localized and less severe osteophytosis, 2 = widespread but moderately severe osteophytosis, and 3 = widespread and very severe osteophytosis. An average of the individual observer scores was calculated for each rat, and the average osteophyte score for each treatment group was then determined.

In vivo micro-MRI. We used a Magnex Scientific in vivo 9.4T high-field micro-MRI to scan the same stifle joint in each rat. Eight weeks after surgery, rats were scanned in vivo under isoflurane anesthesia. The rat was placed supine in the MRI bed, and the right hind limb was extended and secured as described above. Using a flat surface-acquisition coil, we acquired images with anisotropic voxels with a resolution of 137 × 137 × 500 μm. The MRI was operated in a fat-suppressed, spin-echo, T2-weighted scan mode specifically to visualize potential bone marrow lesion–like/edema-like phenomena in the tissue, as previously described (19,20). Each scan was ~15 minutes in duration. Sagittal slices of the medial femoral condyle were selected from each data set, and the epiphyseal water signal was quantified using ImageJ analysis software (NIH Image, National Institutes of Health, Bethesda, MD; online at: http://rsbweb.nih.gov/ij/). The mean visual pixel intensity within a 1.8-mm diameter circular region of interest, anatomically landmarked by the metaphyseal growth plate and the cortical bone margins, was assessed by a single observer (MDJ).

Histologic assessments. After the rats were killed, both the tibia and the femur were dissected from each animal, and the bones were fixed in neutral buffered formalin. After 2 weeks in fixative (with multiple changes), the samples were placed in formic acid–formaldehyde decalcification solution (Cal-EX II; Fisher Scientific) for a further 2 weeks. Decalcified samples were paraffin embedded and sectioned at 6 μm, and the mounted slides were stained with either toluidine blue or

Safranin O to assess cartilage health using a modified Mankin scoring system (21). Slides were assessed in a randomized manner by 2 independent observers (MDJ and GL) who were blinded with regard to study group, and the average of the 2 scores was used for analysis.

Statistical analysis. Results are presented as the mean ± SEM. Statistical analyses were conducted using the SPSS statistics software package (version 13.0; SPSS). The nonparametric Mann-Whitney test was used to compare qualitative histology and osteophyte scores. For the quantitative measures of bone volume, unpaired *t*-tests were used to compare groups. *P* values less than 0.05 were considered significant.

RESULTS

Assays of plasma meloxicam concentrations. Plasma concentrations of meloxicam in the rats were analyzed using an HPLC assay. Meloxicam eluted the column at or near 3.65 minutes and piroxicam, the internal standard, at or near 2.70 minutes, with sufficient resolution between the peaks. Our standard curve correlation R^2 value was equal to 0.999, and the plasma samples, which were obtained at T_{max} as described above from all rats treated with meloxicam (whether alone or in combination with risedronate), had a mean ± SEM meloxicam concentration of 24.13 ± 7.30 μg/ml. Rats that received meloxicam alone had a mean plasma concentration of 33.43 μg/ml, and rats that received combination therapy (meloxicam and risedronate) had a mean plasma concentration of 20.41 μg/ml. The difference in plasma meloxicam concentration between rats treated with meloxicam alone and those treated with a combination of meloxicam and risedronate was not statistically significant.

Results of in vivo micro-CT analysis. The early effects of OA pathogenesis on periarticular bone mass were assessed by measuring the trabecular bone volume percentage in a spherical region of interest within the rat medial femoral condyle (Table 1), using micro-CT data sets. Both bisphosphonates preserved trabecular bony tissue over the 8-week period of developing OA. Relative to the untreated animals with knee triad injury, trabecular bone volume was significantly higher in alendronate-treated animals (+12.1%), and slightly increased in risedronate-treated animals (+4.65%).

Using micro-CT–based bone volume measurements, we did not find any significant thickening or sclerosis of the subchondral bone plate in this rat model of OA up to 8 weeks after knee triad injury (data not shown). However, micro-CT measurements revealed a significantly increased zone of provisional calcification at the primary spongiosum of the growth plates in all animals treated with any bisphosphonate drug, either as a monotherapy or in combination with meloxicam (data not shown). These observations clearly indicate altered

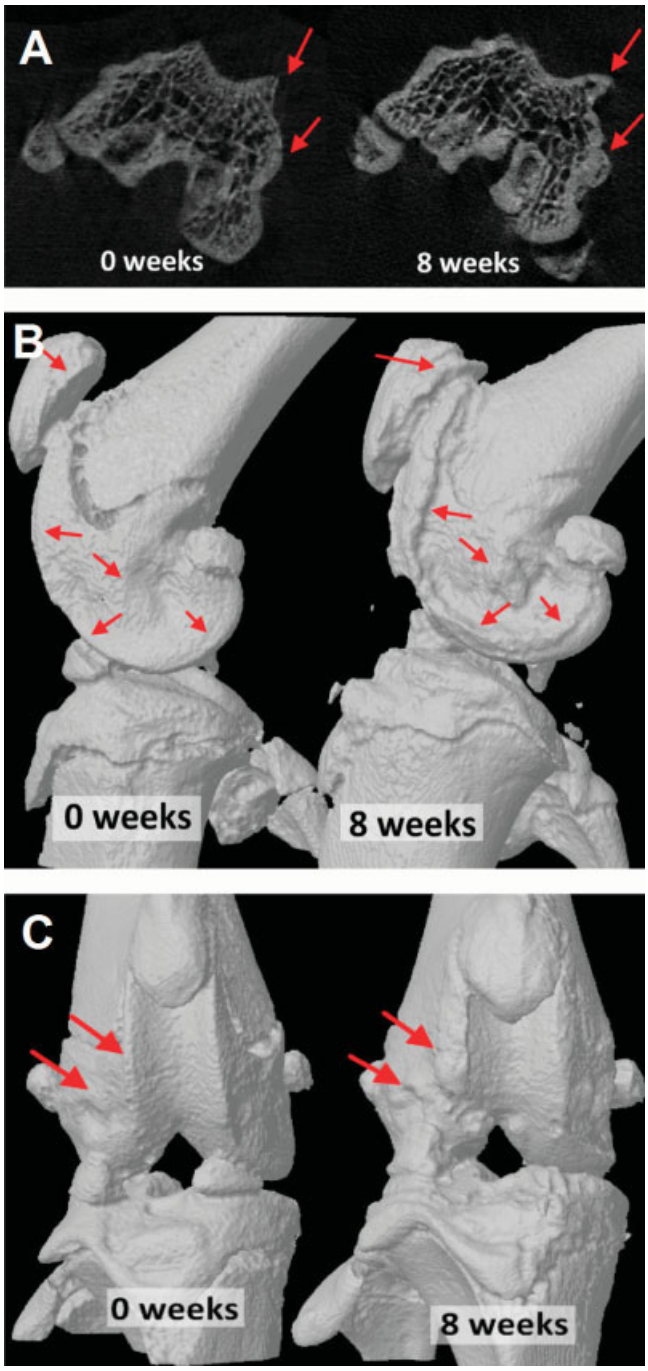

Figure 1. Microfocal computed tomography of osteophyte development in the same knee of an untreated rat 1 day before knee triad injury surgery (0 weeks) and 8 weeks after surgery. **Arrows** indicate areas of osteophyte development. **A,** Transverse slice through the distal femoral epiphysis. **B** and **C,** Three-dimensional renderings of the medial aspect (**B**) and anterior aspect (**C**), illustrating severe osteophyte development.

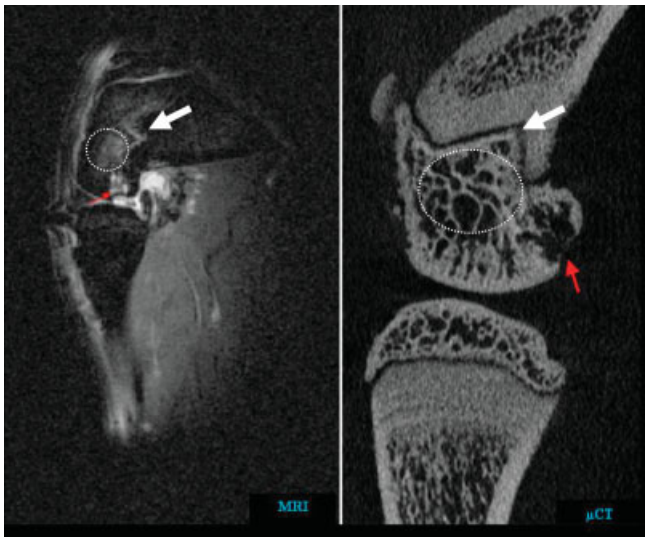

Figure 2. End-stage knee osteoarthritis in the same rat knee viewed by microfocal magnetic resonance imaging (micro-MRI) and microfocal computed tomography (micro-CT). T2-weighted, fat-suppressed MRIs were obtained using a 9.4T system, and micro-CT images were obtained at a resolution of 18 μm. The same subchondral bone cyst (**red arrows**) was observed using both imaging techniques. MRI further revealed an epiphyseal bone marrow lesion–like signal (encircled areas) and delineated the thin metaphyseal growth plate (**white arrows**), while micro-CT showed the associated bone microarchitecture in those anatomic regions at high resolution.

bone remodeling due to the effects of the potent bisphosphonate drugs. However, the effects of bisphosphonates on the nonfused long bone growth plates in the juvenile, growing rats used in this study were not addressed.

Osteophyte growth and severity were assessed qualitatively using 3-D renderings of micro-CT of rat distal femoral bone obtained 8 weeks after surgery (Figure 1 and Table 1). All groups with knee triad injury exhibited osteophytes, with untreated rats with knee triad injury exhibiting severe osteophytosis 8 weeks after surgery (mean score 2.0 [maximum 3.0]). Although osteophyte severity scores for animals treated with alendronate were lower (mean score 1.38 [maximum 3.0]), they were not significantly different from the scores in untreated mice with knee triad injury.

In the second part of our study, temporal evaluations of changes in trabecular bone volume (for all treatment groups) were conducted using in vivo micro-CT in the same rats at time 0 (1 day before surgery) and 4 weeks and 8 weeks after knee triad injury surgery. All randomly assigned groups of rats had an equal trabecular bone volume percentage in the distal femoral epiphysis at time 0. Furthermore, we noted that the trabecular bone volume increased in all groups (both injured and normal controls) from baseline to week 4 and from week 4 to week 8, likely representing the

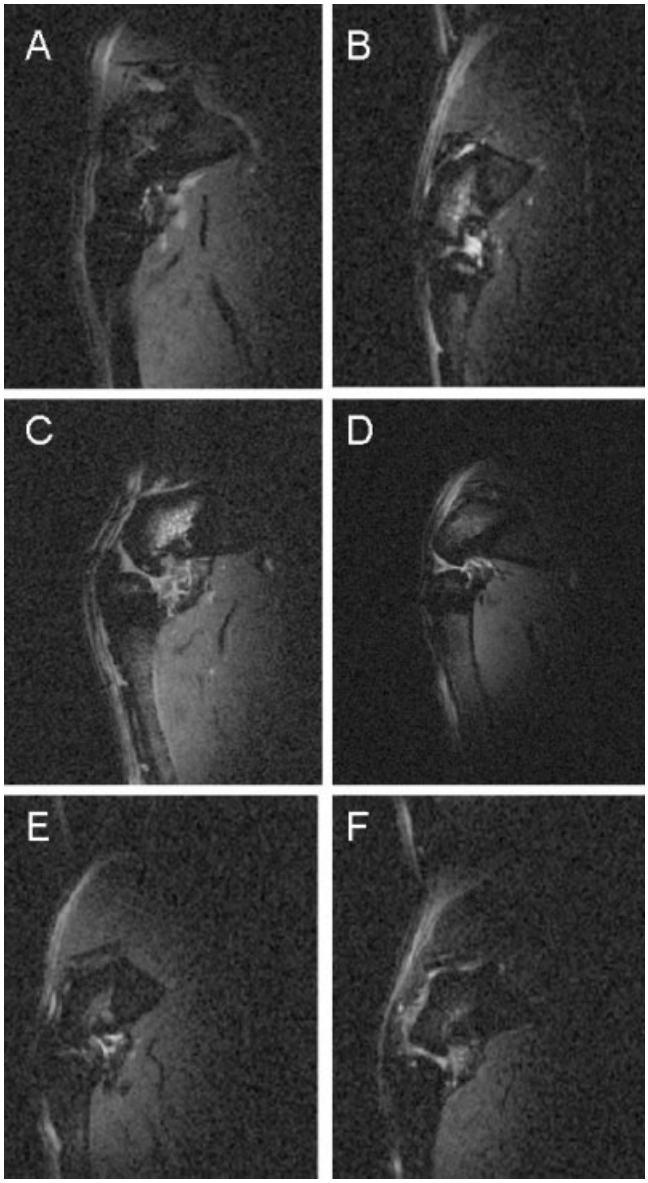

Figure 3. T2-weighted, fat-suppressed microfocal magnetic resonance images of osteoarthritic rat knees 8 weeks after knee triad injury surgery. Images were obtained in the sagittal plane, and the slice transecting the medial condyle of the femur is shown. Images were obtained of **A,** a sham-operated control, **B,** an untreated rat with knee triad injury, **C,** a rat with knee triad injury treated with risedronate, **D,** a rat with knee triad injury treated with alendronate, **E,** a rat with knee triad injury treated with meloxicam, and **F,** a rat with knee triad injury treated with a combination of risedronate and meloxicam. Minimal to no water signal was detected in the sham-operated control rats. Untreated rats with knee triad injury, risedronate-treated rats with knee triad injury, and alendronate-treated rats with knee triad injury showed strong water signals resembling bone marrow lesions in the epiphyseal bone marrow space. Meloxicam as a monotherapy, or in combination with risedronate, reduced the bone marrow lesion–like signal intensity.

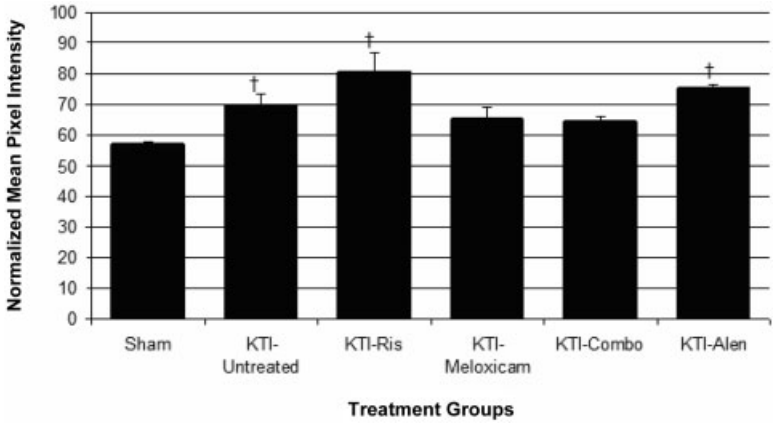

Figure 4. Pixel intensity of a sampled region of epiphyseal trabecular bone, as centered on the metaphyseal growth plate, assessed 8 weeks after surgery to quantify the presence of bone marrow lesions in sham-operated rats, untreated rats with knee triad injury (KTI-untreated), rats with knee triad injury treated with risedronate (KTI-Ris), rats with knee triad injury treated with meloxicam (KTI-meloxicam), rats with knee triad injury treated with a combination of risedronate and meloxicam (KTI-Combo), and rats with knee triad injury treated with alendronate (KTI-Alen). Bars show the mean and SEM. † = $P < 0.05$ versus sham-operated rats.

natural skeletal growth of those rats (data not shown). When we examined the effects of meloxicam, we found an unexpected and significantly increased trabecular bone volume percentage 8 weeks after knee triad injury surgery in the meloxicam-treated rats compared with all other treatment groups (except for the alendronate-treated group) at the same time point ($P < 0.05$) (Table 1). The group treated with a combination of risedronate and meloxicam also exhibited significantly preserved trabecular bone mass compared with untreated rats with knee triad injury ($P < 0.05$). With regard to osteophytosis, the group treated with a combination of risedronate and meloxicam showed a trend toward reduced osteophytosis, but the osteophyte severity score was not significantly different from that in untreated rats with knee triad injury.

Results of in vivo micro-MRI analysis. Using micro-MRI, we visualized the same region in the rat knee joint that was assessed by micro-CT, and we observed increased T2 signal inside the epiphyseal subchondral bone marrow of rats with knee triad injury 8 weeks after surgery (Figure 2). We used the endosteal bone envelope (in the 2-D MRI slices) as anatomic landmarks and constraints for measurements of the epiphyseal bone compartment. Fat-suppressed, T2-weighted micro-MRIs of normal uninjured rats exhibited a discrete, thin T2 signal corresponding to the metaphyseal growth plate. In marked contrast, increased T2 signal was observed at the same location in the epiphyseal bone of untreated rats with knee triad injury, suggestive of a bone marrow lesion–like/edema-like inflammatory abnormality (Figure 3). Significantly increased T2 signal intensity was measured in rats with knee triad injury treated with risedronate and rats with knee triad injury treated with alendronate compared with sham-operated normal controls ($P < 0.05$) (Figure 4). Conversely, the rats with knee triad injury that were treated with the antiinflammatory drug meloxicam, either as a monotherapy or in combination with risedronate, had decreased T2 signal compared with rats with knee triad injury treated with risedronate and untreated rats with knee triad injury 8 weeks after surgery. The T2 signal in rats treated with meloxicam was not statistically different from that in sham-operated normal controls.

Results of histologic analysis. All histologic analysis was performed after rats were killed at the 8-week time point. Each sample was scored using a modified Mankin scoring scheme (Table 1). Eight weeks after surgery, untreated rats with knee triad injury had developed clear indications of cartilage and bone changes indicative of posttraumatic OA. An average modified Mankin score of 5.0 was observed in the untreated group with knee triad injury ($P < 0.05$ versus untreated sham-operated rats). Both animals with knee triad injury treated with risedronate and animals with knee triad injury treated with alendronate showed improved cartilage health compared with the untreated rats with knee triad injury (average score 3.3 for risedronate-treated animals and 3.1 for alendronate-treated animals), although these differences were not statistically signifi-

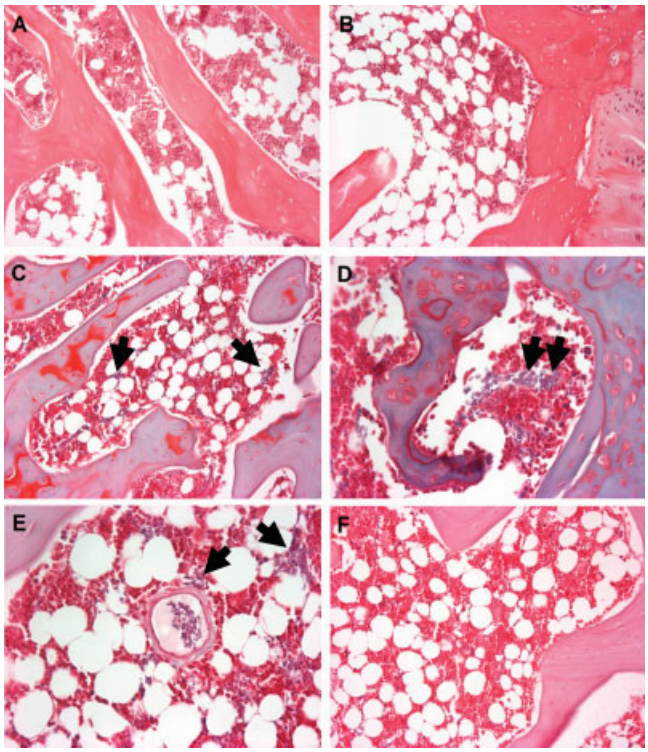

Figure 5. Histologic analysis of trabecular bone from the distal femoral epiphyses of rats that exhibited a bone marrow lesion–like presentation on microfocal magnetic resonance imaging. **A** and **B,** Hematoxylin and eosin (H&E) staining of sections from rats with knee triad injury treated with alendronate (**A**) and rats with knee triad injury treated with risedronate (**B**) (magnification × 20). **C–E,** Safranin O staining of nuclei (red) with fast green counterstaining of erythrocytes (green-blue to purple) of sections from rats with knee triad injury treated with risedronate (original magnification × 20 in **C**; × 40 in **D** and **E**). **F,** Safranin O staining of nuclei (red) with fast green counterstaining of erythrocytes (green-blue to purple) of sections from rats with knee triad injury treated with meloxicam (magnification × 20). There was increased vasodilation of vascular capillaries (**arrows**), resulting in the pooling of erythrocytes (and likely fluid) in the marrow space. Erythrocyte pooling was difficult to discern under routine H&E staining (**A** and **B**), but became powerfully apparent with Safranin O and fast green counterstaining (**C–F**). In the meloxicam-treated samples, the vasodilation appeared reduced, with fewer pools of erythrocytes seen.

cant. Similarly, rats with knee triad injury treated with a combination of meloxicam and risedronate demonstrated a decrease in degradative OA cartilage changes relative to the untreated rats with knee triad injury (average score 3.0). However, meloxicam as a monotherapy did not improve degradative OA cartilage changes in rats with knee triad injury (average score 4.8) compared with untreated rats with knee triad injury.

Histologic examination did not reveal any particular cellular event to which the bone marrow lesion–like phenomenon (seen on micro-MRI in those same bone samples) could be attributed, other than what appeared to be the increased vasodilation of vascular capillaries, which resulted in the pooling of erythrocytes (and likely fluid) in the marrow space. The erythrocyte pooling was difficult to discern under routine hematoxylin and eosin staining (Figures 5A and B) but became powerfully apparent using Safranin O to stain nuclei red, with a fast green counterstain that rendered the erythrocytes green-blue to purple in color (Figures 5C–F). In the meloxicam-treated samples, the vasodilation appeared reduced, with fewer pools of erythrocytes. We did not observe any evidence of leukocytic infiltration over the abundant marrow stromal cells in any of the groups.

DISCUSSION

Despite the prevalence of OA in the aging population, there are no currently approved DMOADs (22). The analgesic acetaminophen is the first-line therapy indicated for OA treatment and is used for pain management, with no known disease-modifying benefits. Several bisphosphonate drugs have previously been shown to have disease-modifying treatment outcomes in OA in animal models (23–25) and humans (26–28). However, those studies had contradictory results regarding the disease-modifying efficacy of bisphosphonate drugs, particularly since most studies in humans involved patients with end-stage OA, rather than individuals at an earlier stage of OA pathogenesis.

In this study, we evaluated the disease-modifying effects of the bisphosphonate drugs risedronate and alendronate and the NSAID meloxicam on the progression of posttraumatic OA in an established rat model (25,29,30). We found that alendronate significantly, and risedronate to some degree, reduced trabecular bone volume loss in the medial femoral compartment compared with untreated rats early after a knee triad injury. These findings were consistent with those of previous studies examining posttraumatic OA in several other animal models (9,31) and were also supported by the results of investigations of risedronate in later-stage human OA (26).

With respect to osteophytosis, risedronate did not reduce or retard bony osteophyte development in rats with knee triad injury, which was consistent with previous results in a rabbit model of OA (9). In contrast, alendronate showed a trend toward reducing the number and severity of osteophytes in the rat knee triad injury model, although the results were not statistically significant compared with the untreated rats with knee triad injury. Our findings are consistent with those of an

earlier study (8) and suggest that alendronate inhibits osteophytogenesis by an alternative mechanism. This mechanism by which alendronate exerts its antiosteophytic activity remains to be determined and is a potential direction for further research.

In the second part of our study, we found that the NSAID meloxicam not only preserved bone volume after a knee triad injury, but also, surprisingly, led to increased bone volume compared with all other treatment groups. As a selective inhibitor of the inducible mediator of inflammation COX-2, meloxicam was previously shown to negatively impact bone healing (32), possibly since COX-2 regulates genes that are critical for osteoblast differentiation (33). This reduced periosteal osteoblastogenesis would therefore likely also reduce subsequent endochondral bone formation. However, the increased epiphyseal trabecular bone volume observed in our study is supported by the observation that disruption of COX-2 gene expression by meloxicam also results in reduced RANKL and osteoprotegerin expression in osteoblasts (34). Thus, a reduction in RANKL levels may have contributed to the maintenance of bone mass in the group of rats with knee triad injury treated with meloxicam. On the other hand, the reduced remodeling observed in all bisphosphonate-treated groups can be attributed to the effects of the potent bisphosphonate drugs. In that scenario, the combination therapy likely did not increase the amount of trabecular bone since bone formation was suppressed by the action of the potent N-containing bisphosphonate drug; this is a well-known consequence of the use of bisphosphonate drugs (35).

It is also important to note that in the present study bone volume percentages appeared to have increased in all groups between the first and second stage of the study (data not shown). These changes could represent differences in the ages of the rat test populations in the first and second parts of the study, since they were supplied based on body mass. Nonetheless, relative to the untreated rats with knee triad injury and untreated uninjured rats in the second part of the study, the rats with knee triad injury treated with meloxicam had a significantly higher bone volume percentage. Interestingly, rats treated with both risedronate and meloxicam did not display any synergistic effects in increased bone volume, but had less bone volume than that observed in rats treated with meloxicam alone. The addition of risedronate may have reduced metaphyseal osteoblastic activity and resulted in reduced bone volume compared with rats treated with meloxicam alone.

Beyond the changes observable on radiography, OA may present pathophysiology that can be detected using physiologic imaging modalities such as MRI. One such phenomenon is a bone marrow lesion (historically termed bone marrow edema), which presents as a high-intensity, diffuse fluid signal within the epiphyses of the femur and tibia of many patients with knee OA (19,20). A bone marrow lesion is identified by increased signal on fat-suppressed, T2-weighted scans, with lower signal contrast to background in T1-weighted scans of the same subchondral bone marrow region. The histologic characteristics of bone marrow lesions in OA remain a subject of controversy. Necrotic bone marrow, bone marrow fibrosis, and occasionally bone marrow edema have been associated with bone marrow lesions in OA (36), and bone marrow lesions are frequently observed in association with the cartilage degradation seen in OA (37). Patients who exhibit bone marrow lesions are more likely to develop severe OA over the subsequent 15–30 months (38), and thus, the early detection of bone marrow lesions with cartilage deterioration may improve the management and treatment of OA.

A major finding of our study was the diffuse, bone marrow lesion–like fluid phase we detected on fat-suppressed, spin-echo, T2-weighted micro-MRIs of the distal femoral epiphyseal marrow space, 8 weeks after injury, in untreated rats with knee triad injury. In stark contrast, a very thin and discrete fluid signal was evidenced in the epiphyses of normal control rats with no surgical injury, suggesting a quiescent basal level of fluid content. In rats with knee triad injury treated with alendronate and rats with knee triad injury treated with risedronate, the diffuse bone marrow lesion–like fluid phase remained 8 weeks after knee triad injury surgery. Conversely, the rats with knee triad injury treated with meloxicam showed a reduced bone marrow lesion–like signal, suggestive of a treatment-based antiinflammatory outcome. The bone marrow lesion–like phenomenon identified using micro-MRI may have a potential application as a measure of OA disease progression and drug efficacy in this posttraumatic rat OA model, and warrants further detailed investigation.

Histologic assessment of the knee joint using a modified Mankin scoring system revealed that the bisphosphonates alendronate and risedronate tended to reduce the progression of OA and maintain cartilage health in this short-term study, although the results were not statistically significant compared with findings in untreated controls with knee triad injury. Those modest chondroprotective effects were consistent with the previously demonstrated positive effects of alendronate therapy in OA (8), and were also supported by the results of investigations of risedronate in later-stage human OA (27,28,39).

In conclusion, our data suggest that bisphosphonates and meloxicam potentially reduce the progression of OA following joint trauma in the rat knee triad injury model and could have additional clinical applications regarding the management of secondary OA. Alendronate and meloxicam were effective in maintaining and increasing bone volume, respectively, while alendronate also displayed some positive effects in inhibiting osteophytosis. Meloxicam alone, or in combination with risedronate, reduced the bone marrow fluid signal at the site of knee triad injury when quantified and compared with signal in sham-operated normal controls. Further research is necessary to elaborate on the precise mechanisms by which bisphosphonates and meloxicam operate in inhibiting the periarticular bone– and fluid-based changes associated with joint damage secondary to trauma. The findings of our study may be applicable in the pharmacologic management of disease following joint damage, secondary to traumatic rupture of the ACL and/or medial meniscus, as is often encountered in sports injuries. In those situations, it may prove feasible to conservatively manage and preserve periarticular bone microarchitecture and retard osteophyte formation by using bisphosphonates and NSAIDs as combination therapy for a discrete therapeutic window immediately after the injury and prior to subsequent surgical joint reconstruction.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Doschak had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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