Chondroprotective effects of zoledronic acid on articular cartilage in dogs with experimentally induced osteoarthritis

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Objective—To assess effects of zoledronic acid on biomarkers, radiographic scores, and gross articular cartilage changes in dogs with induced osteoarthritis.

Animals—21 purpose-bred hound-type dogs.

Procedures—The left stifle joint of each dog was examined arthroscopically to determine initial articular cartilage status, which was followed by cranial cruciate ligament (CrCL) transection to induce osteoarthritis. Dogs were assigned to 3 groups (control group, low dose \[10 \mu g \text{ of zoledronic acid/kg}\], or high dose \[25 \mu g \text{ of zoledronic acid/kg}\]). Treatments were administered SC every 3 months for 1 year beginning the day after CrCL transection. Serum and synovial fluid samples and radiographs were obtained 0, 1, 3, 6, 9, and 12 months after transection. At 12 months, each joint was scored for cartilage defects. Serum and synovial fluid biomarkers of bone and cartilage turnover (bone-specific alkaline phosphatase, type I and II collagen, carboxy-propeptide of type II collagen, and chondroitin sulfate (CS) were analyzed with ELISAs.

Results—The high-dose group had fewer total articular defects and lower severity scores in CrCL-transected stifle joints than did the control group. In addition, the high-dose group had significantly less change in collagenase cleavage of type I or II collagen in the synovial fluid at 1 and 3 months after CrCL transection than did the control group and also had greater changes in bone-specific alkaline phosphatase in synovial fluid at 3 months after CrCL transection than did the control group.


Osteoarthritis is a common debilitating disease with multiple causes and pathways. Osteoarthritis leads to structural change of articular cartilage and bone via biochemical and biomechanical factors. The result is inefficient load sharing of the musculoskeletal system between bone and cartilage, which causes pain. Historically, most studies have been focused on the breakdown of articular cartilage as the hallmark of osteoarthritis because articular cartilage has little capacity to repair itself. However, investigators in a 1986 study proposed that inhomogeneous stiffening of subchondral bone could lead to progression of cartilage lesions in osteoarthritis and may have a role in the initiation of those lesions. Regardless, change to bone or articular cartilage in response to biomechanical or biochemical abnormalities is likely to result in abnormal stresses on the other. Given the potential importance of subchondral bone metabolism in the development and progression of osteoarthritis, investigating methods for modulating changes in subchondral bone may yield new insights into the pathogenesis of osteoarthritis and result in improved treatments for osteoarthritis.

One drug class that has been evaluated and used clinically for its modulatory effects on bone is the bisphosphonate group. Bisphosphonates are analogues of endogenous pyrophosphates that bind strongly to hydroxyapatite, which allows uptake into bone. Bisphosphonates are classified on the basis of their structure. Their structure will affect their affinity to bind to hydroxyapatite and will also affect the potency.

ABBREVIATIONS

- BAP: Bone-specific alkaline phosphatase
- CPII: Carboxy-propeptide of type II collagen
- CrCL: Cranial cruciate ligament
- CS: Chondroitin sulfate
- MMP: Matrix metalloproteinase
- SFA: Société Français d'Arthroscopie
of their mechanism of action. Bisphosphonates reduce bone resorption by acting on recruitment, activity, or life span of osteoclasts through interference with ATP-dependent or mevalonate-synthesis pathways. Bisphosphonates have been used clinically for pathological conditions in bone in which resorption is increased; these conditions include osteoporosis, Paget’s disease, skeletal metastatic disease (including hypercalcemia), and osteogenesis imperfecta. Because subchondral bone is predominantly resorptive in the early stages of osteoarthritis after an injury, bisphosphonates may also prove useful in modulating the progression of osteoarthritis. In particular, these compounds have the potential to inhibit changes in subchondral bone density that may lead to altered stress distribution and subsequent degeneration of articular cartilage. This protection of articular cartilage was detected in a recent study of osteoarthritis in dogs with CrCL transection whereby a bisphosphate, tiludronic acid, was administered at the time of joint stabilization surgery.

Transsection of the CrCL to induce osteoarthritis in dogs has been successfully used to identify biochemical evidence of bone and cartilage turnover. Circulating amino acid sequences that represent specific aspects of cartilage or bone metabolism have been identified and quantitated in serum or synovial fluid. Because these biomarkers can be used to identify changes that occur after injury, they can also be used to monitor affected joints to determine the metabolic effect attributable to treatment with bisphosphonates. Therefore, the objective of the study reported here was to assess results of biomarker assays in combination with results of serial radiographic evaluation and postmortem examination in an effort to thoroughly assess the effects of a potent amino bisphosphonate, zoledronic acid, in dogs undergoing CrCL transection to induce osteoarthritis.

**Materials and Methods**

**Animals**—Twenty-one mixed-breed adult (11 to 24 months old) male hound-type dogs of similar body weight (20 to 35 kg) and body condition were obtained. Skeletal maturity was confirmed on the basis of radiographic evidence of closure of the growth plates. Inclusion criteria specified that all dogs had no history of lameness or stifle joint disease and had no abnormalities detected during physical examination, a CBC, serum biochemical analysis, urinalysis, analysis of synovial fluid obtained from both stifle joints, and radiography of the stifle joints and pelvis. Each dog was housed separately in an individual run (2 × 6.2 m²) throughout the study. Dogs were placed in the runs 3 weeks prior to the beginning of the study to allow them to acclimatize. All procedures were approved by the University of Georgia Animal Care and Use Committee.

**Transsection of the CrCL**—At the beginning of the study (day 0), the left stifle joint of each dog was examined arthroscopically to determine the initial status of the articular cartilage. The CrCL was then completely transected by use of arthroscopic instrumentation. No surgical procedures were performed on the contralateral right stifle joint. Perioperative pain was managed with butorphanol tartrate (0.2 mg/kg, IM), which was administered as part of the preanesthetic medications and again at the time of endotracheal extubation. Postoperative pain was managed with a narcotic (butorphanol), which was administered as a necessary for up to 7 days. After surgery, dogs were returned to their individual runs and remained there for the duration of the study. No exercise protocol was used. Dogs received no other medications, except for a monthly heartworm preventative. Dogs were weighed monthly to ensure they maintained a stable body weight.

**Experimental procedures**—The study was designed as a prospective, placebo-controlled, randomized blinded experimental study. The dogs were allocated by use of a computer-generated randomization table to 3 groups (7 dogs/group). On the day after CrCL transection (day 1), dogs received an SC injection of an inert vehicle (control group) or zoledronic acid at 10 µg/kg (low-dose group) or 25 µg/kg (high-dose group). The same injections were administered to all dogs of the respective groups at a mean ± SD interval of 90 ± 7 days for 12 months (ie, at 3, 6, 9, and 12 months). All dogs were euthanized with an overdose of pentobarbital 12 months after CrCL transection, and necropsy was performed. The investigators were not aware of the treatment administered to each dog.

**Sample collection**—A blood sample (10 mL) was collected from a jugular vein, and a sample of synovial fluid (0.3 to 1.0 mL) was collected (without lavage) from each stifle joint of all dogs on day 0 before CrCL transection (baseline) and 1, 3, 6, 9, and 12 months after transection of the CrCL. All samples were collected between 9 AM and 11 AM. Serum was harvested, and synovial and serum samples were stored at –80°C until assayed.

**Radiographic analysis**—Dogs were sedated and stifle joint radiographs (lateromedial and cranio-caudal views) obtained prior to CrCL transection (baseline) and 1, 3, 6, 9, and 12 months after CrCL transection. Radiographic scores were assigned by 1 investigator (a board-certified veterinary radiologist) in a blinded manner by use of a modified scoring system (Appendix). Gross articular analysis—Cartilage, meniscus, and osteophyte lesions were counted and scored by use of photographs obtained at the time of necropsy. A marker of known length was included in each photograph to allow for differences in magnification. Grades were assigned on the basis of a scoring system described elsewhere. Briefly, grades for cartilage defects were as follows: 1 = superficial fissure, abrasion, or pitting; 2 = partial-thickness erosion or fibrillation; and 3 = full-thickness erosion or defect. The size and location of these defects and of osteophytes were recorded. The area of each cartilage defect was determined, and a severity score was then calculated for each lesion by use of an SFA severity scoring system. Briefly, an SFA severity score was calculated for each lesion by multiplying the area of the lesion (as a percentage of the total surface area) by a weighted severity factor as follows: grade 1, 0.14; grade 2, 0.34; grade 3, 0.65; and grade 4, 1.0; the scores for each lesion were summed to provide
a total joint score. Additionally, the total number of articular defects was determined for each stifle joint.

BAP activity—Bone-specific alkaline phosphatase is a bone-specific isoenzyme of alkaline phosphatase that is synthesized and secreted by osteoblasts. The exact role of BAP is unknown, but it may contribute to calcification of bone matrix. The BAP activity was measured in duplicate in synovial fluid with a monoclonal anti-BAP antibody in a commercially available enzyme immunoassay. Interassay variation for the synovial fluid assay was <15%, and the dynamic range of the assay was 2 to 140 U/L.

Type I and II collagen concentration—Cleavage of the triple helices of type I and II collagen by MMPs results in immunologically distinct neoepitopes at the newly formed carboxy and amino terminal ends of the remaining fragments. Neoepitope concentrations of the carboxy-terminus of the 3/4 peptide of type I and II collagen degradation secondary to collagenase-mediated cleavage were assessed in duplicate in serum and synovial fluid by use of a rabbit polyclonal type I and II collagen antibody (formerly COL2-3/4Cshort) in a 2-step competitive immunoassay. Interassay variation for serum and synovial fluid was <11% and <23%, respectively, and the dynamic range of the assay was 0.03 to 10 µg/mL.

CPII concentration—Carboxy-propeptide of type II collagen is cleaved from the tropocollagen molecule as it becomes incorporated into fibrils and is therefore a potential indicator of type II collagen synthesis. The CPII concentration was determined for each data set. A Bonferroni post hoc test was used to correct for the number of comparisons (α = 0.05/30) and to identify changes within a group or among groups if the F statistic was significant at P < 0.05. Spearman correlation coefficients were calculated for the percentage change from baseline values for data for all groups combined among all of the factors for the following comparisons: BAP activity; type I and II collagen concentration, CPII concentration, and CS 846 concentration in synovial fluid; BAP activity; type I and II collagen concentration, CPII concentration, and CS 846 concentration in serum and total radiographic score; total cartilage score for the CrCL-transected and contralateral stifle joints; and SFA severity scores. A Kruskal-Wallis test with Dunn multiple comparisons was used to determine significant differences in cartilage scores among the control, low-dose, and high-dose groups. Overall value for significance was α = 0.05.

Results

Animals—Each dog underwent complete transection of the CrCL of the left stifle joint. There were no signs of adverse reactions or toxic drug effects attributable to administration of zoledronic acid in any dogs.
throughout the study period. Each dog maintained its body weight within 10% of baseline body weight throughout the study.

**Radiographic analysis**—All groups had a significant increase in radiographic scores for the CrCL-transected stifle joint over the study period, compared with the baseline value (Figure 1). Scores for the nontransected contralateral stifle joint were unchanged over the study period.

**Gross articular analysis**—All CrCL-transected stifle joints had significantly more articular cartilage...
defects, meniscal lesions, and osteophyte changes than did the nontransected contralateral stifle joints (Figure 2). A mean of >1 articular cartilage defect was detected in each nontransected stifle joint, and no meniscal or osteophyte lesions were detected in the nontransected stifle joints.

In CrCL-transected stifle joints, the high-dose group had a lower number of total articular defects and a lower number for the sum of femoral condyle and tibial plateau articular cartilage defects, compared with results for the control group (Table 1). Results for the low-dose group did not differ significantly from results for the control or high-dose groups. For each CrCL-transected stifle joint, the SFA severity score was significantly lower for the high-dose group, compared with the SFA severity score for the control group. The SFA severity score for the low-dose group did not differ significantly from that for the control or high-dose groups. In all CrCL-transected stifle joints, there were no significant differences among the control, low-dose, and high-dose groups for the number of osteophytes. There was no significant difference in severity grades among groups for the femoral condyles, femoral trochlear ridges and patella, or tibial plateaus in CrCL-transected stifle joints. All CrCL-transected stifle joints had medial meniscal damage, but there were no significant differences in the severity of that damage among treatment groups. There was lateral meniscal damage in all dogs of the high-dose group, except for 2. There was a significant difference in the severity of lateral meniscal damage between the control group and the high-dose group. The severity score for the low-dose group did not differ significantly from the severity scores for the other 2 groups.

Serum samples—We did not detect significant differences in percentage change among the control, low-dose, and high-dose groups for serum concentrations of type I and II collagen, CPII, and CS 846 (Figure 3). Percentage change of serum concentrations of CPII was significantly higher than the baseline value in all groups at 12 months after CrCL transection.

Synovial fluid samples—The BAP activity in synovial fluid from the nontransected contralateral stifle joints did not change significantly from baseline values and was not significantly different among treatment groups. Activities of BAP changed significantly from the baseline value throughout the study in the high-dose group (Figure 4). The high-dose group had a greater percentage change than did both the control and low-dose groups at 3 months. The BAP activity for the low-dose group was not significantly different from the BAP activity for the control group at any time point.

Concentrations of type I and II collagen in synovial fluid obtained from the nontransected stifle joints did not change from baseline values and was not significantly different among treatment groups. Concentrations of type I and II collagen in the CrCL-transected stifle joints changed significantly from baseline values in synovial fluid samples obtained from the control group at 1 to 9 months after transection. The change from baseline values was significantly greater for the control group than for the low-dose group from 1 to 9 months after transection and was significantly grea-

Table 1—Gross articular analysis of the CrCL-transected left stifle joint for 21 purpose-bred male hound-type dogs allocated to 3 groups (7 dogs/group) that received an SC injection of an inert vehicle (control group) or zoledronic acid at 10 µg/kg (low-dose group) or 25 µg/kg (high-dose group) on the day after CrCL transection and 1, 3, 6, 9, and 12 months after CrCL transection.

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*Values reported represent mean ± SD. †Value differs significantly (P < 0.05; Kruskal-Wallis test with Dunn multiple comparisons) from the value for the control group. ‡The SFA severity score was calculated for each lesion by multiplying the area of the lesion (as a percentage of total surface area) by a weighted severity factor (grade 1, 0.14; grade 2, 0.34; grade 3, 0.65; and grade 4, 1.00); the scores for each lesion were summed to provide a total joint score.
er than the change for the high-dose group at 1 and 3 months after transection (Figure 4).

For both the CrCL-transected and nontransected stifle joints, there was little percentage change in synovial fluid concentrations of CPII over time. Only the high-dose group had a significant increase in CPII concentration in synovial fluid samples obtained from the nontransected stifle joints at 12 months, compared with the baseline concentration. There was no significant difference in CPII concentration among groups for both the CrCL-transected and nontransected stifle joints (Figure 4).

The CS 846 concentrations in synovial fluid did not change from baseline concentrations in the CrCL-transected and nontransected stifle joints. Synovial fluid concentrations of CS 846 did not differ significantly among treatment groups (Figure 4).

Correlations—Significant correlations were detected between biomarkers and radiographic findings when data for all groups were combined. These bio-

![Figure 3](image-url)

**Figure 3**—Percentage change in serum concentrations of type I and II collagen (A), CPII (B), and CS 846 (C) over time for dogs in each of 3 groups. Values represent percentage change from the baseline value. There was not a significant effect of dose on percentage change for type I and II collagen (P = 0.631), CPII (P = 0.952), or CS 846 (P = 0.115) concentrations or of time on type I and II collagen (P = 0.106) or CS 846 (P = 0.164) concentrations or on the treatment-by-time interaction of type I and II collagen (P = 0.483), CPII (P = 0.185), or CS 846 (P = 0.081) concentrations. However, there was a significant effect of time on percentage change for CPII concentration (P < 0.001). See Figure 1 for remainder of key.

![Figure 4](image-url)

**Figure 4**—Percentage change in BAP activity (A and B), type I and II collagen concentration (C and D), CPII concentration (E and F), and CS 846 concentration (G and H) over time in synovial fluid obtained from the CrCL-transected (A, C, E, and G) and contralateral nontransected (B, D, F, and H) stifle joint of dogs in each of 3 groups. For the CrCL-transected stifle joints, there was a significant (P ≤ 0.001) effect of dose, time, and the treatment-by-time interaction on BAP activity and type I and II collagen concentrations as well as a significant effect of time on CS 846 concentration (P = 0.020); however, there was not a significant effect of dose, time, or the treatment-by-time interaction on CPII concentration (P = 0.379, 0.337, and 0.300, respectively) or dose or treatment-by-time on CS 846 concentration (P = 0.412 and 0.312, respectively). For the nontransected stifle joint, there was a significant effect of dose on CS 846 concentration (P = 0.049), but there was no significant effect of dose on the remaining variables (P ≥ 0.072 for all analyses). In addition, there was not a significant effect of time on type I and II collagen concentrations (P = 0.154), but there was a significant effect of time on BAP activity (P = 0.003), CPII concentration (P = 0.003), and CS 846 concentration (P = 0.031). There was no significant effect of the treatment-by-time interaction on BAP activity (P = 0.484) or type I and II collagen concentrations (P = 0.506); however, there was a significant effect of the treatment-by-time interaction on CPII (P < 0.001) and CS 846 (P = 0.049) concentrations. Within a time point, value for the high-dose group differs significantly (P < 0.0017) from the control group value. Within a time point, value for the high-dose group differs significantly (P < 0.0017) from the low-dose group value. See Figures 1 and 3 for remainder of key.
markers included synovial fluid BAP activity ($r = 0.495$; $P < 0.001$), serum CPII concentration ($r = 0.334$; $P < 0.001$), and synovial fluid CS 846 concentration ($r = 0.243$; $P = 0.011$). There were no significant correlations of any of the biomarkers with gross articular lesion scores when all groups were combined.

**Discussion**

In the present study, a potent bisphosphonate, zoledronic acid, had chondroprotective effects on articular cartilage that could be identified via the combination of macroscopic and biochemical changes on samples obtained 1 year after CrCL transection. This protective effect was identified primarily as a reduced number of cartilage lesions in the high-dose (25 µg/kg) group, compared with the number of cartilage lesions in the control group. This may have been partially mediated through regulation of collagenase activity, given that concentrations of type I and II collagen were significantly reduced in the synovial fluid obtained from bisphosphonate-treated dogs.

Results for several studies on osteoarthritis in animals provide the rationale for use of bisphosphonates or other bone antiresorptive drugs for modulating subchondral bone remodeling to reduce the progression of osteoarthritis disease. Effects of zoledronic acid on articular cartilage are presumably expected to be a secondary process mediated through alteration of subchondral bone metabolism and structure during the onset and progression of osteoarthritis. These cartilage-sparing effects were not detected in a previous study, despite the fact there was reduced turnover and resorption of subchondral bone following CrCL transection in dogs. That study was concluded after only 12 weeks, so it is possible that differences in cartilage scores may have been detectable had it been continued for 1 year after CrCL transection, similar to the study reported here. Another study in which investigators examined the effect of bisphosphonate treatment administered concurrently with surgical stabilization of the stifte joint 1 month after CrCL transection supports the theory of bisphosphonates modulating subchondral bone remodeling to reduce the progression of osteoarthritic disease. It appears that even if bisphosphonates have an effect on the subchondral bone within the first few months after injury, macroscopic effects on cartilage will not be evident until months later.

As indicated by the results of the present study, the dose of bisphosphonate administered determined whether there was a chondroprotective effect. The number of total cartilage lesions and SFA severity score were lower in the high-dose group, compared with results for the control group, but there was no difference between the control group and low-dose group. However, there did not appear to be an effect for sparing of any particular region, even though there was evidence of region-specific changes in bone mineral density following administration of zoledronic acid at 25 µg/kg in another study. In humans and domestic animals, there is evidence that bisphosphonates cause a dose-dependent reduction in bone turnover. Analysis of results of these studies indicates that increasing the dose causes a greater and longer lasting effect on bone. In dogs with CrCL transection in the aforementioned study, administration of zoledronic acid at a high dose (25 µg/kg), but not at a low dose (10 µg/kg), prevented significant decreases in bone mineral density seen in control dogs starting at 1 month after transection. However, there is evidence that high doses of zoledronic acid may actually increase microdamage over time and lead to potential fractures. It has been suggested that part of the effect of the bisphosphonate dose may be related to the degree of bone turnover in the patient and the interval between doses. In the present study, all dogs received treatments at the same intervals, and it is unlikely that individual variation in bone turnover would have factored into our results, considering that there was no difference in the experimentally induced injury among groups and no evidence of regional protection within the joints. Therefore, it appears that the dose of zoledronic acid was the most critical factor, and a higher dose presumably allowed for more binding to hydroxyapatite.

Abnormal metabolic turnover of articular cartilage after CrCL transection in dogs has been clearly confirmed by use of biomarkers of osteoarthritis in serum and synovial fluid. Similar to results for those studies, synovial fluid was superior to serum for examination of the acute stage after a single joint injury in the present study. In addition, we obtained similar results over time for type I and II collagen, CPII, and CS 846 concentrations in synovial fluid. After CrCL transection, there is an increase in collagenase cleavage of types I or II collagen that starts within the first month after CrCL transection. The cause for this increase from 1 to 9 months after CrCL transection is presumed to be an increase in activated collagenases, primarily MMP-13 and -1, in response to joint injury and inflammation. In the study reported here, both the low- and high-dose groups had less collagenase cleavage (type I and II collagen) than did the control group. This finding is similar to results for other studies in which bisphosphonates have been found to affect diseased chondrocytes and decrease synthesis and gene expression of MMP-13. It has also been postulated that reduction in collagenase values may be as a result of altered MMP production by bisphosphonate-affected osteoclasts. The exact mechanism for action of the bisphosphonates on MMP activities is not known, but the mechanism may be related to cation chelation. These findings suggest the likelihood that some of the cartilage-sparing effects identified in the dogs of the present study may have been attributable, in part, to a decrease in circulating MMP activity, especially given that there was no evidence that bisphosphonate increased CPII synthesis, which has been reported in another study. However, we did not measure collagenase activities directly; there is only an indication that collagenases were present in the synovial fluid because of exposure of the neoepitopes in the triple helix of collagen. Although the type I and II collagen assay can be used to identify cleavage of both type I collagen (primarily found in bone) and type II collagen (primarily found in articular cartilage), immunostaining with the COL2-3/4E8 antibody has been used to detect differences within osteoarthritic cartilage, compared with results in cartilage explants obtained from clinically normal humans.
It should be further mentioned that a limitation of the present study with regard to the biomarkers used was that there was no attempt to correct the concentrations on the basis of synovial fluid volume or clearance rates. In addition, some biomarkers could be used to identify changes that occurred over time, which thereby suggested the role of various biochemical pathways. Ultimately, their concentrations did not correlate with the pathological changes identified, which made it difficult to interpret their role in nonstabilized stifle joints. Further studies need to be performed to delineate these effects.

Transection of the CrCL in dogs results in an unstable joint that continually incites further inflammation and damage. Therefore, it is not surprising that radiographic scores increased from baseline values for all 3 groups in the present study. In addition, results of radiographic analysis in the study were consistent with previous observations that the radiographic score does not necessarily correlate with articular cartilage degeneration in a number of species. One of the biggest reasons for this may be that the osteophyte count is a major determinant for the total radiographic score, and the number of osteophytes was unchanged by bisphosphonate administration in the present study, which is similar to results of another report. Osteophytes can be the result of mechanical and humoral factors such as transforming growth factor-β, and transforming growth factor-β1 expression in diseased chondrocytes is unaffected by bisphosphonate administration.

One potential explanation for the increase in total radiographic score may have been the BAP activity in a joint. Similar to results of a previous study, we detected a significant correlation between synovial fluid BAP activity and total radiographic score in the present study. The BAP activity in the nontransected stifle joint was unchanged, which is similar to results reported in other studies. Therefore, it appears that there is a local source of BAP within the synovial fluid of a CrCL-transected joint. Bone-specific alkaline phosphatase is a marker of early osteoblastic activity, so the local source of BAP may be reactive osteoblasts within the osteophytes. It is unknown how a high dose of bisphosphonate would affect BAP activity. The increased values in the present study represented an absolute increase in osteoblastic activity or possibly a relative increase in activity resulting from an alteration in the balance of osteoclastic and osteoblastic activity secondary to zoledronic acid inhibition of osteoclast activity. Further studies need to be performed to provide information on this relationship.

Furthermore, zoledronic acid was administered immediately after CrCL transection to create joint instability but before osteoarthritis was established. This may have provided a different pathophysiological situation than would be seen for established osteoarthritis. Thus, results of the present study must be considered in that context, and additional studies should be performed to enable us to assess the effects of zoledronic acid in joints with established osteoarthritis. Additionally, although there was no clinical evidence of toxicoses in the present study, bisphosphonate-related osteonecrosis, specifically of the mandible and maxilla, has been reported after long-term use and should be evaluated in future studies.

In the present study, a high dose (25 μg/kg) of the bisphosphonate zoledronic acid administered after CrCL injury resulted in a chondroprotective effect. However, progression of the radiographic lesions continued despite this potential preventive treatment.

References

17. Wise LM, Waldman SD, Kasra M, et al. Effect of zoledronic acid immediately after CrCL transection to create joint instability but before osteoarthritis was established. This may have provided a different pathophysiological situation than would be seen for established osteoarthritis. Thus, results of the present study must be considered in that context, and additional studies should be performed to enable us to assess the effects of zoledronic acid in joints with established osteoarthritis. Additionally, although there was no clinical evidence of toxicoses in the present study, bisphosphonate-related osteonecrosis, specifically of the mandible and maxilla, has been reported after long-term use and should be evaluated in future studies.

In the present study, a high dose (25 μg/kg) of the bisphosphonate zoledronic acid administered after CrCL injury resulted in a chondroprotective effect. However, progression of the radiographic lesions continued despite this potential preventive treatment.

### Appendix

Determinants scored in a modified radiographic scoring system of stifle joint osteoarthritis severity in dogs.

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Type</th>
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<tr>
<td>Femoral troclear groove periaricular osteophytes</td>
<td>Lateral or medial condylar periaricular osteophytes</td>
</tr>
<tr>
<td>Femoral subchondral sclerosis</td>
<td>Femoral condylar remodeling</td>
</tr>
<tr>
<td>Femoral condylar remodeling</td>
<td>Subchondral cystic luescenes</td>
</tr>
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<td>Sesamoid periaricular osteophytes</td>
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<tr>
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<td>Femoral intercondylar notch osteophytes</td>
</tr>
<tr>
<td>Femoral intercondylar notch osteophytes</td>
<td>Periarthritic osteophytes in proximal aspect of the tibia</td>
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<tr>
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<td>Subchondral sclerosis in proximal aspect of the tibia</td>
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<td>Subchondral cystic lesions in proximal aspect of the tibia</td>
</tr>
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<td>Tibial plateau osteophytes</td>
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<tr>
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<td>Joint effusion and capsular thickening</td>
</tr>
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<td>Intra-articular mineralized osseous fragments</td>
</tr>
<tr>
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<td>Meniscal mineralization</td>
</tr>
<tr>
<td>Meniscal mineralization</td>
<td>Intercondylar avulsion fracture fragments</td>
</tr>
</tbody>
</table>

*Each determinant was scored on a scale of 0 to 3 (0, absent; 1, mild; 2, moderate; or 3, severe).*