

# Licofelone Reduces Progression of Structural Changes in a Canine Model of Osteoarthritis Under Curative Conditions: Effect on Protease Expression and Activity

MAXIM MOREAU, CHRISTELLE BOILEAU, JOHANNE MARTEL-PELLETIER, JULIE BRUNET, STEFAN LAUFER, and JEAN-PIERRE PELLETIER

**ABSTRACT. Objective.** We investigated the effectiveness of licofelone, a combined 5-lipoxygenase and cyclooxygenase inhibitor, on structural changes in the anterior cruciate ligament (ACL) experimental dog model of osteoarthritis (OA) under therapeutic conditions. The effect of drug treatment on the expression and activity of metalloproteases in the OA cartilage was also studied.

**Methods.** The cranial cruciate ligament of the right stifle joint was surgically sectioned in 14 dogs to create OA lesions. Of these dogs, 7 received placebo treatment and served as OA controls, while 7 were treated with licofelone 2.5 mg/kg twice daily for an 8-week period, starting 4 weeks after surgery. At necropsy, macroscopic evaluations were made of the size of osteophytes and the severity of cartilage lesions on femoral condyles and tibial plateaus. Collagenase and other metalloprotease activity levels in cartilage were measured. Levels of gene expression of matrix metalloprotease (MMP-1), MMP-13, cathepsin K, and ADAMTS-5 were quantified by RT-PCR.

**Results.** Licofelone treatment reduced the development of osteophytes and size of cartilage lesions on the femoral condyles and on the tibial plateaus ( $p < 0.04$ ). Drug treatment also significantly decreased collagenase ( $p < 0.02$ ) and metalloprotease ( $p < 0.04$ ) activities, as well as the levels of gene expression of MMP-1 ( $p < 0.01$ ), MMP-13 ( $p < 0.05$ ), cathepsin K, and ADAMTS-5 ( $p = 0.01$ ).

**Conclusion.** Under therapeutic conditions licofelone showed the ability to reduce the progression of structural changes in experimental dog OA. This beneficial effect is likely mediated through decrease in the synthesis of a number of catabolic factors, including proteolytic enzymes, involved in cartilage breakdown. (First Release May 1, 2006; J Rheumatol 2006;33:1176–82)

## Key Indexing Terms:

LICOFELONE DOG OSTEOARTHRITIS CARTILAGE MATRIX METALLOPROTEASES

The structure of an arthrodial joint is susceptible to being altered by the presence of osteoarthritis (OA), which can greatly affect the patient's quality of life and functional capacities, with troubling clinical results. Characteristics of OA commonly seen in practice include symptoms and signs such as joint pain and swelling, and structure changes such as osteophytes, cartilage erosion, and subchondral bone remodeling<sup>1</sup>. The extent of bone changes has been documented to reach trabecular bone metabolism<sup>2–5</sup>. There is an undeniable

need for drugs to alleviate the clinical signs of OA, and ultimately to reduce or stop progression of the disease<sup>6</sup>.

The developmental pathology observed in OA can be defined as often being mechanically driven, although it is a chemically mediated inflammatory disease<sup>1,7,8</sup>. In OA, the joint cartilage is subjected to a degenerative process when the chondrocytes fail to produce functional extracellular matrix (ECM) components in a manner to palliate for the excessive tissue breakdown and depletion occurring under supraphysiologic mechanical forces. In addition, the chondrocytes themselves react to the force-related destruction of ECM components, and subsequent loss of physical properties, by elaborating inflammatory mediators<sup>1,7</sup>. Among the mediators responsible for OA cartilage destruction and joint inflammation, a number of proinflammatory cytokines such as interleukin 1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and IL-6 seem to play a predominant role<sup>1,7,9</sup>. The synthesis of ECM components by chondrocytes in attempts at repair is inhibited by the presence of these cytokines<sup>9,10</sup>. Further, the cartilage degradation is excessively amplified as chondrocytes, together with synoviocytes, are modulated to produce a number of proteolytic zinc- and calcium-dependent enzymes, referred to as the matrix metalloproteases (MMP)<sup>11</sup>. In this family, the collagenases (MMP-1 and MMP-13) and stromelysin (MMP-3)

From the Osteoarthritis Research Unit, University of Montreal Hospital Centre, Notre-Dame Hospital, Montreal, Quebec, Canada.

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M. Moreau, MSc, The Companion Animal Research Group, Faculty of Veterinary Medicine, University of Montreal, St. Hyacinthe, Quebec; C. Boileau, PhD, Postdoctoral Fellow; J. Martel-Pelletier, PhD, Professor of Medicine, Director, Osteoarthritis Research Unit; J. Brunet, PhD, Research Associate, Osteoarthritis Research Unit; S. Laufer, PhD, Head, Department of Pharmaceutical Chemistry/Medicinal Chemistry, Eberhard-Karls-University, Tübingen Institute of Pharmacy, Tübingen, Germany; J.-P. Pelletier, MD, Professor of Medicine, Director, Osteoarthritis Research Unit, University of Montreal Hospital Centre.

Address reprint requests to Dr. J.-P. Pelletier, Osteoarthritis Research Unit, University of Montreal Hospital Centre, Notre-Dame Hospital, 1560 Sherbrooke St. East, Montreal, Quebec H2L 4M1, Canada. E-mail: dr@jppelletier.ca

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have been identified as playing significant roles in the enzymatic digestion of major ECM components, including type II collagen and large aggregating proteoglycan<sup>1,11,12</sup>. In OA, the aggrecans seem to be predominantly degraded by ADAMTS-5, an aggrecanase of the adamalysin thrombospondin family<sup>13,14</sup>.

Licofelone is a promising antiarthritic drug that offers dual inhibition of 5-lipoxygenase (5-LOX) and cyclooxygenases (COX-1 and COX-2), thus inhibiting leukotriene and prostaglandin production<sup>15,16</sup>. The effect of combined COX and 5-LOX inhibition has deepened the knowledge of eicosanoid-related pathways that intervene in OA disease progression<sup>17-20</sup>. Hence, licofelone has exerted a positive effect on OA structural changes, as perceived in experimental OA, acting on cartilage, synovial membrane, and subchondral bone when administered under prophylactic conditions<sup>2,20</sup>. Suppression of eicosanoid synthesis and the subsequent reduction in levels of proteolytic enzymes and chondrocyte apoptosis as well as cytokines in OA synovium may explain the mechanisms by which licofelone protects the joint against damages in the canine model of OA<sup>20-22</sup>.

Under clinical conditions, the treatment of OA is initiated when structural changes have already taken place. These include cartilage lesions and quite often a certain level of synovial inflammation. It would therefore seem most relevant to explore the disease-modifying effect of a drug under conditions that are as close as possible to the real-life scenario. We examined the effect of licofelone on the progression of experimental OA structural changes and main pathophysiological pathways under therapeutic conditions.

## MATERIALS AND METHODS

**Experimental groups.** Fourteen adult crossbred dogs (2–3 years old, weighing 20–25 kg) were used in this study. Surgical sectioning of the anterior cruciate ligament (ACL) of the right knee through a stab wound was performed on all dogs as described<sup>20</sup>. Prior to surgery, the animals were anesthetized intravenously with pentobarbital sodium (25 mg/kg) and intubated. The dogs received a fentanyl patch (75 µg) starting 24 h before surgery for the management of operative and postoperative pain. The fentanyl treatment was repeated every 72 h and was supplemented with injection of oxymorphone (0.1 µg/kg subcutaneously), if required. Administration of the analgesic drugs followed guidelines established for perioperative pain management and approved by the Institutional Ethical Committee. Following surgery, the dogs were kept at a housing farm [approved by the Canadian Council on Animal Care (CCAC)] where they were free to exercise in a large pen. The dogs were randomly separated into 2 treatment groups. The groups were balanced according to age, sex, and weight. Seven dogs received placebo treatment (encapsulated methylcellulose) to serve as OA controls and 7 other dogs received encapsulated licofelone, 2.5 mg/kg twice daily (8 AM and 4 PM) for 8 weeks at total daily doses of 5 mg/kg/day, beginning 4 weeks following surgery throughout the duration of the study. Technical personnel were blinded to the treatment groups. All dogs were euthanized 12 weeks after surgery. The actual dosage of licofelone has been shown to be within therapeutic range<sup>20</sup>. The drug treatment in this study and the previous study was found to be very well tolerated with no significant side effects. The weight of the dogs was stable throughout the duration of the study. The Institutional Ethical and Scientific Committee approved the study protocol in accord with the guidelines of the CCAC.

**Macroscopic grading.** Immediately after the dogs were sacrificed, the right knee of each dog was dissected and placed on ice, and the synovial fluid was

aspirated. Each knee was examined by 2 independent observers (JPP and CB) who were blinded to the treatment groups, and gross morphologic changes were recorded as described<sup>23</sup>. The degree of osteophyte formation was graded by measuring the maximal width (in mm) of the spur on the medial and lateral femoral condyles with the use of a digital caliper (Digimatic Caliper; Mitutoyo, Kawasaki, Japan); these values recorded from each dog were considered separately for the purpose of statistical analysis. The cartilage changes on the medial and lateral femoral condyles and tibial plateaus were graded separately using a dissecting microscope (Stereozoom; Bausch & Lomb, Rochester, NY, USA).

The macroscopic changes were graded<sup>23</sup> as follows: (1) changes to the articular surface area were measured, with results expressed in mm<sup>2</sup>; and (2) the depth of erosion was graded on a scale of 0–4, with 0 = surface appears normal, 1 = minimal fibrillation or a slight yellowish discoloration of the surface, 2 = erosion extending into the superficial or middle layers, 3 = erosion extending into the deep layers, and 4 = erosion extending to the subchondral bone.

**Histologic grading.** Histologic evaluation was performed on sagittal sections of cartilage from the lesional areas of each femoral condyle and tibial plateau as described<sup>23</sup>. Specimens were dissected, fixed in TissuFix #2 (Chaptel, Montreal, QC, Canada), and embedded in paraffin for histologic evaluation. Serial sections (5 µm) were stained with safranin O and with hematoxylin-fast-green-safranin O (Chroma, Münster, Germany). Severity of the OA lesions was graded by 2 independent observers (JPP and CB) on a scale of 0–32, using the histologic/histochemical scale of Sakakibara, *et al*<sup>24</sup>. This scale was used to evaluate the severity of OA lesions based on the loss of safranin O staining (scale 0–4), cellular changes (scale 0–12), changes in the tidemark (scale 0–3), and structural changes (scale 0–10), in which 0 = normal cartilage structure and 10 = complete disorganization and formation of pannus (scale 0–3). Scoring was based on the most severe histologic changes within each cartilage lesion.

**Proteolytic enzyme activity assay.** Representative cartilage samples from the lesional areas of the femoral condyles and tibial plateaus were carefully dissected from the underlying bone. For each dog, the pooled cartilage was used to measure collagenase and general metalloprotease activity and the remainder was used for polymerase chain reaction (PCR) analysis.

Extraction of proteolytic enzymes from the cartilage was carried out as described<sup>23</sup>. The tissue was sliced and homogenized in extraction buffer (50 mM Tris HCl, 10 mM CaCl<sub>2</sub>, 2 M guanidine HCl, 0.05% Brij-35, pH 7.5). The mixture was stirred overnight at 4°C and then centrifuged (18,000 g for 30 min at 4°C). The supernatant was extensively dialyzed (48 h at 4°C) against an assay buffer (50 mM Tris HCl, 10 mM CaCl<sub>2</sub>, 0.2 M NaCl, 0.05% Brij-35, pH 7.5) using Spectrapor 4 dialysis tubing with a 12 kDa cutoff (Spectrum Medical Industries, Los Angeles, CA, USA).

The collagenase activity in the tissue extract was measured using telopeptide-free type I collagen from rat tail tendon acetylated with <sup>14</sup>C-acetic anhydride<sup>25</sup>. One hundred microliters of <sup>14</sup>C-collagen suspension (12,000 disintegrations/min) were incubated under the following conditions: (1) with a 100 µl aliquot of tissue extract in the presence of 1 mM aminophenylmercuric acid (APMA); and (2) with a 100 µl aliquot of tissue extract containing 1 mM APMA and 25 mM EDTA, to serve as a blank. Each solution was incubated 48 h at 30°C, after which each was centrifuged at 12,000 g for 15 min at 4°C. Radioactivity contained in the supernatant was determined using a beta scintillation counter (Beta Rack, Model 1218; LKB, Stockholm, Sweden). The total enzymatic activity was expressed in units per gram of tissue wet weight (ww), in which 1 unit corresponded to the hydrolysis of 1 µg of substrate/h at 30°C. More than 90% of the collagenase activity was inhibited by EDTA.

The general metalloprotease activity in the cartilage extract was measured by the method of Chavira, *et al*<sup>26</sup>, using azocoll (Calbiochem-Behring, San Diego, CA, USA) as substrate. One hundred microliters of the azocoll suspension were incubated in a manner similar to the collagenase activity, except that 1 mM 1,10-phenanthroline served as the blank. After incubation (48 h at 37°C) this solution was centrifuged (12,000 g for 15 min at 4°C) and the optical density of the supernatant was determined by spectrophotometric analy-

sis. Protease activity was expressed in units per gram of tissue (ww) in which 1 unit corresponded to the hydrolysis of 1  $\mu$ g of substrate/h at 37°C. The azo-coll-digesting activity in the extracts was inhibited > 90% by 1,10-phenanthroline.

#### Real-time quantitative PCR analysis

**Extraction of total RNA from cartilage.** Total RNA was extracted directly from the cartilage. Cartilage from lesional areas of the condyles and the plateaus was pooled to allow a sufficient amount of tissue to be processed for RNA extraction. Cartilage was suspended in a Trizol buffer (Invitrogen, Life Technologies, Burlington, ON, Canada) and processed as described<sup>22</sup>. The purified RNA was quantified by spectrophotometry.

**Quantification.** Quantification of gene expression levels for MMP-1, MMP-13, cathepsin K, and ADAMTS-5 was carried out by real-time quantitative PCR with the GeneAmp 5700 sequence detection system (Applied Biosystems, Foster City, CA, USA) using the QuantiTect SybrGreen PCR kit (Qiagen, Mississauga, ON, Canada), as described<sup>22</sup>. The sense (S) and anti-sense (AS) oligonucleotides used for PCR studies were as follows: 5'-AAG ACA GGT TCT ACA TGC GC-3' (S) and 5'-AGT CAG CTG CTA TCA TCT GG-3' (AS) for MMP-1; 5'-TTG GTC AGA TGT GAC ACC TC-3' (S) and 5'-ATC GGG AAG CAT AAA GTG GC-3' (AS) for MMP-13; 5'-AGG TGG ATG AAA TCT CTC GG (S) and 5'-TTC TTG AGT TGG CCC TCC AG (AS) for cathepsin K; 5'-GTC GGG ACC ATA TGT TCT C (S) and 5'-TGA TGG TGG CTG AAG TAC AC (AS) for ADAMTS-5; and 5'-AGG CTG TGG GCA AGG TCA TC-3' (S) and 5'-AAG GTG GAA GAG TGG GTG TC-3' (AS) for GAPDH.

The data were collected and processed using GeneAmp 5700 sequence detection system software, with results presented as a threshold cycle ( $C_t$ ). Plasmid DNA containing the target gene sequences were used to generate the standard curves. A DNA standard curve for each gene was prepared and used in the quantitative PCR reactions. The  $C_t$  was then converted to the number of molecules, and the results for each sample were calculated as the ratio of the number of molecules of the target gene to the number of molecules of GAPDH. The primer efficiencies for the test genes were the same as for the GAPDH gene.

**Statistical analysis.** Values are expressed as the mean  $\pm$  SEM or as median (range). Statistical analysis was performed using Wilcoxon rank-sum tests or Mann-Whitney U test. P values  $\leq$  0.05 were considered significant.

## RESULTS

**Macroscopic findings.** Osteophytes were present on the femoral condyles of all the OA control dogs, and their widths were similar to those observed in the licofelone-treated dogs ( $4.4 \pm 0.4$  mm vs  $3.3 \pm 0.6$  mm, respectively; Table 1).

All the dogs had cartilage lesions on the femoral condyles and tibial plateaus (Figure 1). Lesions observed on tibial plateaus were larger and more severe than those on the femoral condyles (Table 1). Compared to the OA control group, the licofelone-treated dogs had smaller cartilage

lesions on both femoral condyles and tibial plateaus (Figure 1). The treated dogs exhibited a marked and statistically significant reduction in the size of their lesions on tibial plateaus ( $p < 0.04$ ) compared to OA control dogs (Table 1). The reduction of lesion size on the condyles of treated dogs was also pronounced (48%); however, these differences did not reach statistical significance. The severity (grade) of lesions on condyles and plateaus was similar in the licofelone-treated group compared to the OA control group.

**Microscopic findings.** The global severity of the histological scores of OA changes in the lesional areas of the condyles ( $17.4 \pm 3.1$  and  $14.1 \pm 2.3$ ) and plateaus ( $18.1 \pm 2.5$  and  $14.7 \pm 1.8$ ) was similar in the control and licofelone-treated groups, respectively). However, there was an interesting trend toward the severity of lesions being less marked in the licofelone-treated dogs than the placebo-treated dogs on the lateral femoral condyles ( $6.6 \pm 1.5$  and  $10.6 \pm 2.4$ , respectively) and on the medial tibial plateaus ( $9.2 \pm 2.0$  and  $13.0 \pm 2.7$ ; Figure 2).

**Proteolytic enzyme activity.** In both groups, collagenase and general metalloprotease enzyme activity was quantified in the OA cartilage (Table 2). Licofelone-treated dogs showed a significantly lower level of proteolytic activity for both collagenase ( $p < 0.02$ ) and general metalloprotease activity ( $p < 0.04$ ) compared to placebo-treated dogs.

**Gene expression.** The level of expression of MMP-1, MMP-13, and ADAMTS-5 was found to be significantly lower in the licofelone-treated dogs compared to OA control dogs (Figure 3). The results were similar with cathepsin K, where the level of gene expression was also reduced by treatment with licofelone.

## DISCUSSION

Licofelone treatment has previously been shown to be capable of reducing the development of experimental OA under prophylactic conditions<sup>20</sup> and improving the clinical symptoms of OA in both dogs<sup>27</sup> and humans<sup>28</sup>. We observed that the drug is also effective at reducing the progression of already existing lesions. The effect of the treatment was correlated with a reduction in the expression and synthesis of key proteolytic enzymes that have been found to be involved in OA cartilage degradation<sup>1,2</sup>.

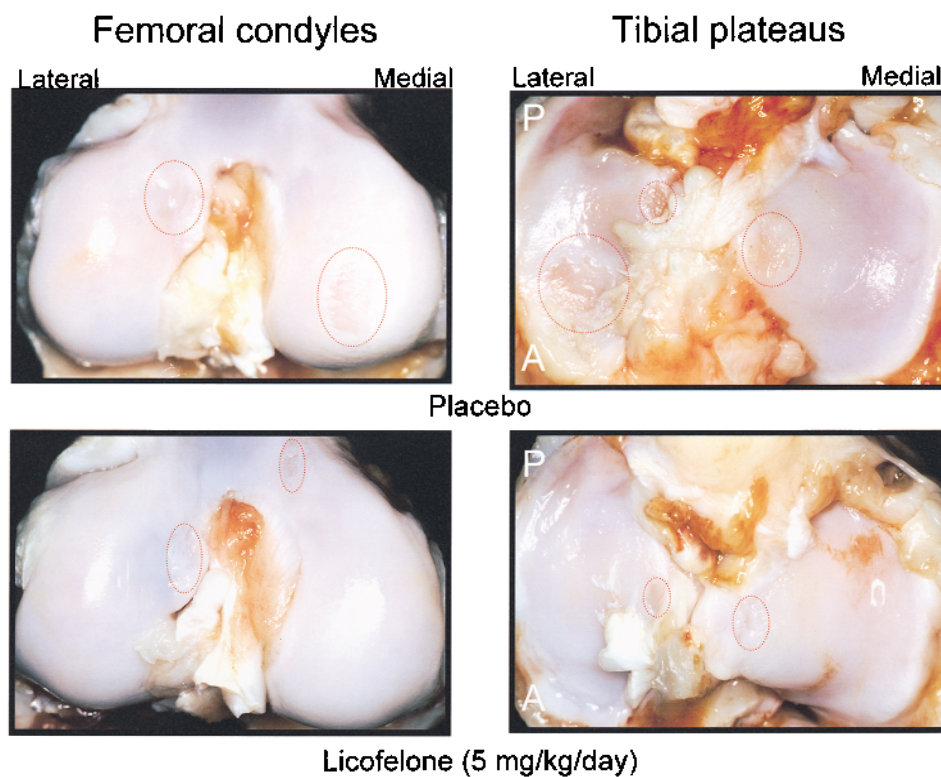
The experimental ACL dog model of OA is a most relevant model with which to study the pathophysiology of the disease

Table 1. Macroscopic findings of dog OA cartilage. Values are mean  $\pm$  SEM.

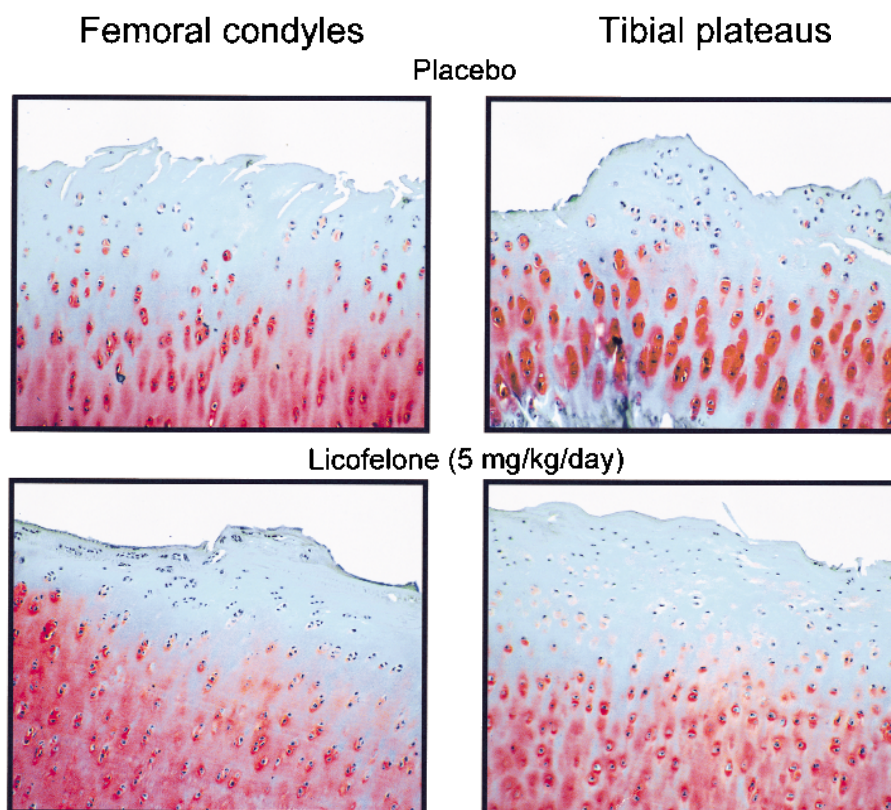
Groups (n)	Osteophytes, Femoral Condyles, Width, mm <sup>2</sup>	Macroscopic Cartilage Lesions			
		Femoral Condyles Size, mm <sup>2</sup>	Severity*	Tibial Plateaus Size, mm <sup>2</sup>	Severity*
OA control (7)	$4.4 \pm 0.4$	$18.9 \pm 5.3$	$1.6 \pm 0.4$	$41.8 \pm 5.2$	$2.2 \pm 0.3$
Licofelone 5 mg/kg/day (7)	$3.3 \pm 0.6$	$9.0 \pm 2.5$	$1.4 \pm 0.3$	$31.0 \pm 2.2^{**}$ ( $p < 0.04$ )	$1.9 \pm 0.2$

\* Highest grade observed in each dog. \*\* Statistically significant compared to OA control.





**Figure 1.** Macroscopic appearance of OA cartilage from femoral condyles and tibial plateaus of placebo-treated and licofelone-treated dogs at 12 weeks. Erosion and pitting (areas indicated by circles) of condyles and plateaus were evident in placebo-treated dogs. A decrease in the severity of lesions on the condyles and plateaus was seen in licofelone-treated dogs. A: anterior, P: posterior.



**Figure 2.** Representative sections of OA cartilage from the lateral femoral condyles and medial tibial plateaus of placebo-treated and licofelone-treated dogs (stained with hematoxylin-fast green-safranin O; original magnification  $\times 100$ ).

Table 2. Proteolytic enzyme activity (mean  $\pm$  SEM) of joint stifle cartilage in a canine model of OA.

Groups (n)	Collagenase Activity, Units/g of Tissue ww*	Metalloprotease Activity, Units/g of Tissue ww*
OA control (7)	49.7 $\pm$ 4.7	234.3 $\pm$ 14.1
Licofelone 5 mg/kg/day (7)	34.0 $\pm$ 5.0* ( $p < 0.02$ )	189.0 $\pm$ 16.2** ( $p < 0.04$ )

\* Values are expressed in units by gram of tissue wet weight. \*\* Statistically significant compared to OA control.

and the development of new therapeutic interventions. Several studies have demonstrated and validated the usefulness of the model in testing new drugs that target the major pathophysiological pathways of OA<sup>20,23,29-32</sup>. Licofelone, a dual inhibitor of 5-LOX and COX, has been studied to determine its effects on OA progression as well as its potential to modulate the major disease pathways<sup>18,20-22</sup>. In the experimental OA dog model, the drug was effective at reducing the development of lesions when administered prophylactically, starting immediately following the surgery. Under these conditions, licofelone was shown to reduce the expression and synthesis of metalloproteases and other proteolytic enzymes such as ADAMTS-4 and -5 and cathepsin K<sup>22</sup>. Moreover, the drug treatment was also very effective at reducing the level of IL-1 $\beta$  in OA synovium<sup>20</sup>. In addition, treatment with licofelone was shown to reduce the remodeling of subchondral bone<sup>22</sup>, a phenomenon

likely to play an important role in the pathophysiology of the disease.

In our study, licofelone reduced the progression of already established lesions. The effect was noted on both condyles and plateaus. The extent of protection on lesion size was comparable to that observed in our previous study done under prophylactic conditions<sup>20</sup>. The effect on the grade (depth) of lesions, however, was less marked. This is an interesting finding, as it suggests at least 2 hypotheses: that the lesions were stabilized at the level of severity present at the time drug treatment began, or, on the other hand, that the drug treatment is more effective at reducing extension of the lesions over the cartilage surface than it is at stopping the penetration of lesions throughout the cartilage thickness. Similar findings have been reported by our group in this model under similar conditions with tenidap, an antiinflammatory-anticytokine drug<sup>23,33</sup>. The absence of any significant effect of the drug treatment on osteophyte progression was somewhat expected, as no such effect of the drug was found under prophylactic conditions<sup>20</sup>.

Treatment with licofelone was also found to markedly reduce the level of a number of proteolytic enzymes known to be involved in OA cartilage degradation<sup>20,22</sup>. Both MMP-1 and MMP-13 are believed to be among the most important enzymes in degrading the native type II collagen in articular cartilage<sup>1,11</sup>. MMP-1, which is predominantly distributed in the superficial layers of diseased cartilage, would seem more

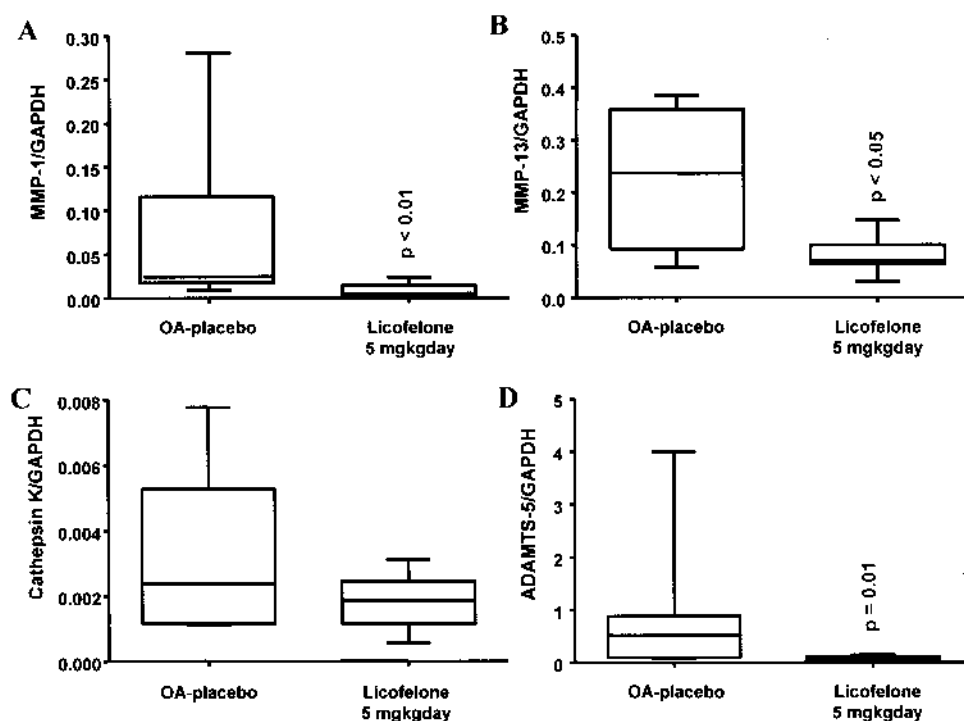


Figure 3. Gene expression (mRNA level) by real-time quantitative PCR analysis of (A) MMP-1, (B) MMP-13, (C) cathepsin K, and (D) ADAMTS-5. Data are expressed as median and range; boxes represent the 1st and 3rd quartiles, the line within the box represents the median, and lines outside the box represent the spread of values. P values were compared to the OA placebo group; Mann-Whitney U test.

likely to be responsible for inducing the erosion of cartilage surface. MMP-13, on the other hand, is distributed in the superficial as well as the deep layers of OA cartilage. Therefore, in addition to enhancing the effect of MMP-1 in the erosion of cartilage, this enzyme is believed to induce remodeling of the deeper zones of cartilage, including the calcified zone. Moreover, in early experimental dog OA, MMP-13 was shown to be involved in the resorption/remodeling of subchondral bone and the calcified cartilage zone<sup>22</sup>. Thus MMP-1 and MMP-13 play major roles in the destruction and remodeling of the entire cartilage thickness as well as the adjacent bone. The reduction in the level of these 2 enzymes by licofelone treatment offers strong support to its disease-modifying action. The exact mechanisms by which licofelone suppresses MMP synthesis are not completely understood. However, recent findings from our laboratory indicate that the drug can inhibit IL-1 $\beta$ -induced MMP-13 synthesis in chondrocytes through the suppression of the p38/AP-1 pathway<sup>34</sup>. It is also possible that the inhibition of MMP synthesis *in situ* in experimental OA may have been related at least in part to the inhibition of IL-1 $\beta$  synthesis by the drug treatment<sup>20</sup>. Studies have shown that licofelone, through the inhibition of leukotriene-B<sub>4</sub> synthesis, was a potent inhibitor of cytokine synthesis, both *in vitro* and *in vivo* in OA synovial membrane<sup>18,20</sup>.

Cathepsin K is a proteolytic enzyme that has been found in increased amounts in OA tissues, including the articular cartilage and subchondral bone. It is believed that through its degrading activity on both collagen type II and aggrecan, this enzyme plays a predominant role in OA cartilage matrix degradation<sup>35</sup>. Since cathepsin K has also been identified in OA calcified cartilage, this enzyme is likely involved not only in the genesis of OA cartilage erosion but also in the progressive thinning of the calcified cartilage observed during the evolution of the disease. Moreover, as cathepsin K is a potent enzyme capable of inducing bone resorption, its presence could explain the thinning of the subchondral plate and resorption of the trabecular bone seen in early osteoarthritic lesions. Therefore our finding that licofelone could reduce cathepsin K synthesis is most interesting. It may not only explain the reduction in the cartilage destruction observed under the actual experimental conditions, but also indicate the potential of this drug to reduce or stop the progression of already existing lesions. This is of particular importance in the context of a drug to be used under clinical conditions where structural damage is present at the time that treatment is initiated.

Finally, our findings on the effect of licofelone on the inhibition of ADAMTS-5 clearly support recent publications<sup>13,14</sup> showing the predominant role of this enzyme in OA cartilage aggrecan degradation. This enzyme has also been the subject of studies that have highlighted its role in the pathophysiology of OA and the importance of a number of growth factors and cytokines in its regulation *in situ*<sup>36-40</sup>. Therefore the inhibition of ADAMTS-5 by licofelone is an additional factor that

may have contributed to the protective effect of the drug on cartilage lesions in our study.

In summary, our study provides new and interesting information about the potential role of licofelone in the treatment of OA. The drug was found to be capable of reducing the progression of already existing OA lesions, thereby bringing a new dimension to the potential of the treatment to be effective at the clinical stage of the disease. Moreover, we found that licofelone is capable of reducing the synthesis of a number of proteolytic enzymes, thereby blocking important OA pathophysiological pathways even when they were well established in the disease process. However, given the relatively small number of dogs included in this study and its short duration, one should exercise caution in drawing any firm conclusions on the potential of the drug in the context of treatment of the natural disease. Therefore, the question remains to what degree these findings can be extrapolated into clinical OA, which essentially means determining whether licofelone could exert disease-modifying activity in humans. A phase III study of the effect of the drug on the progression of structural changes in patients with knee OA is under way and should shed some light on this most interesting and challenging question.

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