Pro-Apoptotic Effects of Tepoxalin, a Cyclooxygenase/Lipoxygenase Dual Inhibitor, on Canine Synovial Fibroblasts

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ABSTRACT. Canine osteoarthritis occurs frequently and causes secondary synovitis. Administration of nonsteroidal anti-inflammatory drugs (NSAIDs) is one of the major therapeutic options for pain management of joint diseases. Tepoxalin has an unique property as an NSAIDs that suppresses both cyclooxygenase and lipoxygenase. The purpose of this study was to evaluate antiproliferative effects of tepoxalin on cultured canine synovial cells. Cytotoxic effects of tepoxalin, carprofen, meloxicam and AA-861 on cultured canine synoviocytes were evaluated by MTT colorimetric assay. Apoptosis was detected by morphological observations with Giemsa or annexin V/Hoechst 33342 staining and by the inhibition of caspase-3 activity with N-Ac-Asp-Glu-Val-Asp-CHO (Ac-DEVD-CHO). Cytotoxic effects of tepoxalin were evident in comparison with the effects of carprofen or meloxicam. The same tendency of cytotoxicity was observed when 5-lipoxygenase was inhibited by AA-861. The morphological findings and contradictory effects of Ac-DEVD-CHO with regard to the cytotoxicity proved the proapoptotic effects of tepoxalin. In conclusion, tepoxalin might control osteoarthritic synovitis by inducing apoptosis in proliferating synoviocytes, while most NSAIDs that selectively inhibit cyclooxygenase-2 most likely would not suppress synovial proliferation.

KEY WORDS: apoptosis, canine, osteoarthritis, synoviocytes, tepoxalin.

Osteoarthritis (OA) is one of the most common joint diseases in many kinds of mammals. Approximately 20% of adult dogs could have osteoarthritic joints [14]. OA has been defined as an inherently noninflammatory disorder of movable joints characterized by degeneration of articular cartilage and by the formation of new bone at joint surfaces and their margins [24]. Recent studies indicated that the inflammatory reaction of the synovium could play an important role in the degenerative process of the osteoarthritic structure [22, 27]. In osteoarthritic joints, the reactive synovium shows hypertrophy and hyperplasia with an increased number of lining cells and also infiltration of inflammatory cells, revealing that inflammation would certainly be participating in the process [21]. Severe synovitis, which is consistently seen in rheumatoid arthritis pannus, is one of the common findings in some pathologies of canine OA [9, 13]. Pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1, play a central role in OA. Activated synoviocytes are one of the main sources of cytokines, and an increase in synoviocytes worsens inflammation of the synovium and other structures of the entire joint [25, 28]. Thus, inducing apoptosis of the activated and increased numbers of synoviocytes is thought to be a part of the anti-inflammatory effects that improve osteoarthritic conditions [3].

In small animal practice, nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used as a symptomatic treatment for OA [24, 26]. It tends to be believed that NSAIDs promote anti-inflammatory and analgesic effects by inhibition of cyclooxygenase (COX), which catalyses the conversion of arachidonic acid to inflammatory mediators such as prostaglandins (PGs) and thromboxanes [24, 29]. Most of the favorable effects of NSAIDs in OA patients, however, seem to be limited to relieving pain and not direct reduction of joint inflammation [26].

In recent years, COX-2 inhibitors, a type of NSAID, such as carprofen, meloxicam, firocoxib and deracoxib have been widely used to treat canine OA as symptomatic osteoarthritis agents, which minimize fatal adverse effects, such as gastrointestinal hemorrhage [18]. Tepoxalin is currently used as one of the other options for canine OA treatment and has been mentioned as an unique NSAID that acts as a dual inhibitor, inhibiting both COX and lipoxygenase (LOX) [12]. The harmful gastrointestinal effects of tepoxalin are thought to be as same as the effects of meloxicam. [7]. Anti-inflammatory properties related to LOX inhibition are likely to suppress reactive synovitis in OA affected joints. However, knowledge of the clinical efficacy of the anti-inflammatory properties of tepoxalin is very limited in the literature [1, 11, 20, 23].

The objectives of the present study were to evaluate the magnitude and manner of cytotoxicity of tepoxalin in cultured osteoarthritic synoviocytes *in vitro*.

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MATERIALS AND METHODS

Cell culture: Canine fibroblast-like synoviocytes (CFLSs) were freshly isolated from inner layers of joint capsules removed from 3 dogs undergoing surgery for cranial cruciate ligament rupture. All the dogs suffered from secondary OA and had an enlarged synovium, from which a portion was to be removed. In order to use this tissue in this study, informed consent was obtained from the owners of the patients. Synovial tissue was dissected out, minced and incubated for 4 hr at 37°C in 5% CO_2 with serum-free Dulbecco's Modified Eagle's Medium (DMEM; Gibco-BRL, Paisley, U.K.) supplemented with 4 mg/m*l* of collagenase type IA (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and antibiotics (100 IU/m*l* of penicillin and 0.1 mg/m*l* of streptomycin). Cells were suspended in DMEM with 10% fetal bovine serum (FBS; Biomedical Inc., Aurora, OH, U.S.A.), 100 IU/m*l* penicillin and 0.1 mg/m*l* streptomycin. Cells were incubated at 37°C in 5% CO_2 to obtain monolayer adherent cultures. At confluence, cells were trypsinized and passaged. Cells between the second and fifth passage were used for experiments. Cell viability, assessed by trypan blue exclusion test, always exceeded 90%.

In vitro cell viability assay: The viability of cells was evaluated using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay [4]. In a 96-well flat-bottomed microplate (Corning, Corning, NY, U.S.A.), 2×10^4 cells/well of CFLS were incubated for 24 hr at 37°C. CFLSs were treated with different concentrations of meloxicam (Wako), carprofen (Wako), tepoxalin (Zubrin, Takeda Schering-Plough Animal Health, Osaka, Japan) and AA-861 (Wako) for 24 hr. To determine the activity of caspase-3, 100 μM of a caspase-3-specific inhibitor, N-Ac-Asp-Glu-Val-Asp-CHO (Ac-DEVD-CHO, Wako), was added 1 hr prior to exposure to tested agents. To investigate the involvement of the 5-LOX pathway, 10 μM of a 5-LOX metabolite, 5-oxoETE (Cayman, Ann Arbor, MI, U.S.A.), was added with tepoxalin and AA-861. At the end of the treatment, 10 μl of MTT solution (10 mg/m*l*, Wako) was added. After 4 hr of incubation, the cultures were centrifuged (760 × *g*, 10 min). The supernatants were removed, and 100 μl of solution buffer (47.5 μl of deionized distilled water with 47.5 μl of N-N-dimethylformamide and 20 mg of sodium dodecyl sulfate, pH 4.7) was added to each well and oscillated for 1 min. The light absorbance of each well was measured with a wavelength of 570 nm. The results were presented as the average of triplicate samples. All test drugs were dissolved in dimethyl sulfoxide (DMSO). Final concentrations of DMSO in all tested solutions were less than 0.1%.

Morphological evaluation by optical microscopic analysis: On 8-well chamber slide glasses (Nalge Nunc International, Rochester, NY, U.S.A.), 1×10^5 cells/well of CFLSs were seeded and incubated for 24 hr at 37°C in 5% CO_2 with serum-free DMEM. Solutions of drugs were added to each well, and the cells were then incubated for another 24 hr. Final concentrations of test drugs were 100 μM for tepoxalin, 100 μM for carprofen, 100 μM for meloxicam and 10 μM for AA-861. Slides were air-dried, fixed with methanol
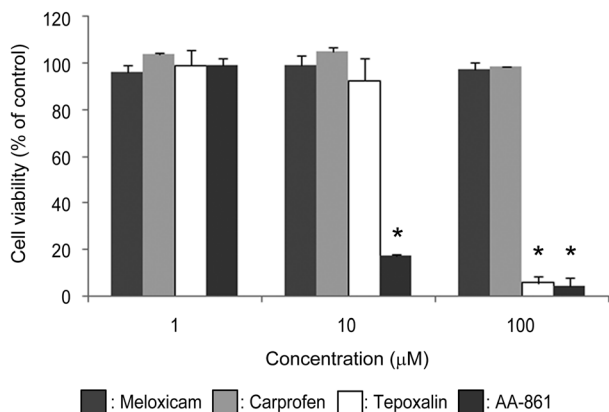

Fig. 1. Effects of tepoxalin, carprofen, meloxicam and AA-861 on CFLSs. CFLSs were incubated for 24 hr with each tested drug (1, 10 and 100 μM). Cell viability was estimated by MTT assay. Values are shown as means ± SD from triplicate cultures. Cytotoxic effects of tepoxalin and AA-861 were evident in comparison with those of the control. *: *P*<0.01.

for 1 min and stained with Giemsa solution (Merck, Whitehouse Station, NJ, U.S.A.). The morphological changes in the nucleus and cytoplasm of cells were observed with an optical microscope.

Evaluation of apoptosis induction by fluorescence microscopical analysis: Treated cells were washed with cold phosphate-buffered saline twice. Cells were suspended in buffer (10 mM HEPES, 140 mM NaCl and 2.5 mM $CaCl_2$, pH 7.4) at a concentration of 1×10^6 cells/m*l*. Ten microliters of annexin V, Alexa Fluor® 488 conjugate (Lonza, Walkersville, MD, U.S.A.) and 1 μM of Hoechst 33342 solution (Sigma-Aldrich Co., St. Louis, MO, U.S.A.) were added to the cells, and the cells were incubated for 15 min at room temperature. The nuclear morphology of the cells and cell membrane were visualized using a fluorescence microscope (FV500, Olympus, Tokyo, Japan).

Data analysis and statistics: Each data set was expressed as the mean ± SD. All data were statistically analyzed by performing the Tukey-Kramer test using a commercial statistical software (StatView 5.0, SAS institute Inc., Cary, NC, U.S.A.) to determine significant differences between groups. *P* values less than 0.05 were considered significant.

RESULTS

Cell viability: The viability of CFLSs treated with each drug is shown in Fig. 1A significant decrease (*P*<0.01) in cell viability was observed with 100 μM of tepoxalin in comparison with cells treated with vehicle only (0.1% DMSO). In contrast, carprofen and meloxicam had no significant effects on cell viability at all examined concentrations. Supplementation with AA-861 reduced cell viability by much more than supplementation with tepoxalin.

Apoptosis-related morphological changes in CFLSs: As shown in Fig. 2, morphological changes were observed in
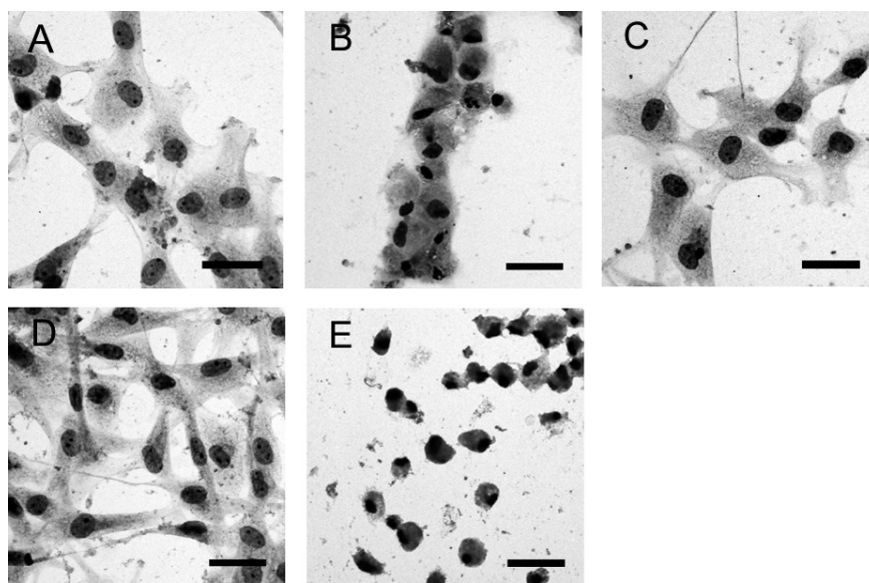
Fig. 2. Morphological changes of CFLSs. A: untreated control. B: CFLSs treated with 100 μM of tepoxalin. C: CFLSs treated with 100 μM of carprofen. D: CFLSs treated with 100 μM of meloxicam. E: CFLSs treated with 10 μM of AA-861. Severe aggregation in the nucleus and shrinkage and strong staining in the cytoplasm were observed in both tepoxalin- and AA-861-treated cells. No morphological changes were found in either carprofen- or meloxicam-treated cells. Scale bar=50 μm.
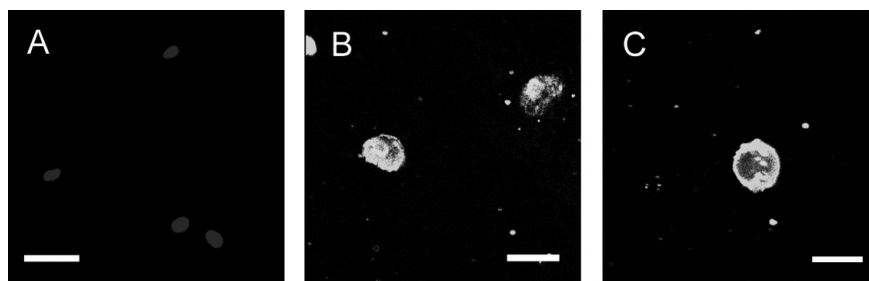

Fig. 3. Annexin V-FITC and Hoechst 33342 double staining for apoptosis detection in tepoxalin- and AA-861-treated cells. Differential interference contrast images of CFLSs were obtained by confocal laser microscopy with annexin V-FITC and Hoechst 33342 double staining. A: untreated control. B: CFLSs treated with 100 μM of tepoxalin. C: CFLSs treated with 10 μM of AA-861. An annexin V-positive stained cellular membrane and contracted nucleus were observed in tepoxalin- and AA-861-treated CFLSs. Scale bar=20 μm.

Giemsa-stained CFLSs treated with tepoxalin or AA-861. Severe shrinkage in the nucleus and strong staining in the cytoplasm were evident after culture with either drug. There were no morphological changes found in CFLSs treated with carprofen or meloxicam compared with the control. Annexin V-FITC/Hoechst 33342 double staining was performed to detect cells in the early phase of apoptosis after treatment (Fig. 3). Control cells were not stained by annexin V and were stained only by Hoechst 33342 (Fig. 3A). Annexin V-positive CFLSs were observed after treatment with 100 μM of tepoxalin (Fig. 3B) and 10 μM of AA-861 (Fig. 3C). Shrinkage in the nucleus was detected in tepoxalin- and

AA-861-treated cells by Hoechst 33342 nuclear staining (Fig. 3B and 3C). Fragmentation of CFLSs nucleus was not detected in the control and tepoxalin- and AA-861-treated cells.

Change of cell viability by caspase-3 inhibition: Tepoxalin and AA-861 proved to be able to induce apoptosis in CFLSs at certain doses after inhibition of caspase-3. Some of this proapoptotic effect induced by tepoxalin or AA-861 was partially inhibited by Ac-DEVD-CHO (Fig. 4). The survival rate of CFLSs treated with 20 μM of tepoxalin was 7.8 $\pm$ 5.7%. The rate was then significantly improved to 44.1 $\pm$ 16.2% by supplementation with 100 μM of Ac-DEVD-CHO
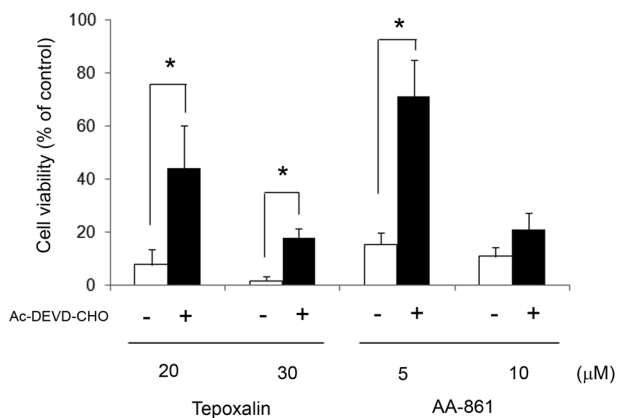
Fig. 4. Changes in cell viability after inhibition of caspase-3. CFLSs were treated with tepoxalin or AA-861 at the indicated concentrations together with (black bars) or without (open bars) 100 μM of Ac-DEVD-CHO for 24 hr. Values of treated CFLSs are shown as percentages of those of control cells. Proapoptotic effects of tepoxalin and AA-861 were significantly inhibited by Ac-DEVD-CHO. Data are means ± SD (n=3). *: P <0.05.
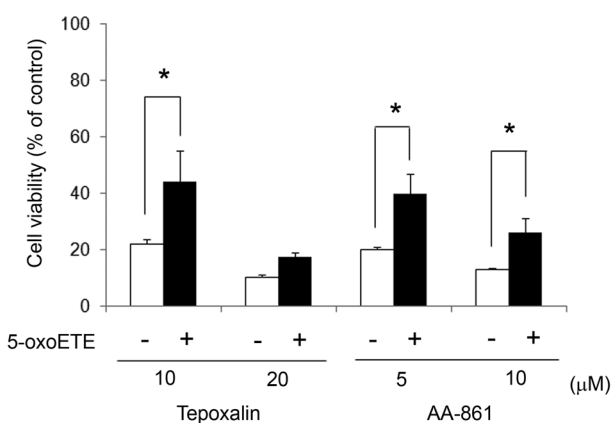
Fig. 5. Changes in cell viability after supplementation with 5-oxoETE. CFLSs were treated with tepoxalin or AA-861 at the indicated concentrations together with (black bars) or without (open bars) 10 μM of 5-oxoETE for 24 hr. Values of treated CFLSs are shown as percentages of those of control cells. Proapoptotic effects of tepoxalin and AA-861 were significantly inhibited by 5-oxoETE. Data are means ± SD (n=3). *: P<0.05.

(P<0.01). The survival rate of CFLSs in 30 μM of tepoxalin (1.9 ± 1.7%) was likewise raised to 18.1 ± 3.4% by Ac-DEVD-CHO (P<0.01). Treatment with 10 μM of AA-861 reduced the cell viability of CFLSs to 15 ± 4.6%. The rate was then significantly improved to 71.4 ± 13.6% by 100 μM of Ac-DEVD-CHO (P<0.01). The survival rate of CFLSs in 20 μM of AA-861 (10.9 ± 3.5%) was raised to 21.1 ± 6.1% by Ac-DEVD-CHO (P<0.05).

Change in cell viability after 5-LOX metabolite treatment: To determine whether the cytotoxic effects tepoxalin and AA-861 were related to 5-LOX inhibition, CFLSs were treated with 5-oxoETE, a metabolic product of 5-LOX. As a result, the cytotoxic effects of tepoxalin and AA-861 were significantly inhibited (Fig. 5). In tepoxalin-treated CFLSs, cell viability was approximately doubled by supplementation with 5-oxoETE (P<0.05). The same tendency was observed for AA-861-treated CFLSs.

DISCUSSION

Osteoarthritis occurs frequently in certain populations of dog, and could cause severe synovitis [22]. An inflamed synovium is one of the main sources of cytokines [15] and needs to be controlled for prevention of catastrophic sequelae. Thus, inducing apoptosis of activated and increased numbers of synoviocytes is thought to be a part of the anti-inflammatory effects that modify OA conditions [17]. In the present study, carprofen and meloxicam, which are recognized as selective or preferential COX-2 inhibitors, and tepoxalin, which is one of the COX/LOX dual inhibitors, were investigated to evaluate their cytotoxic effects on CFLSs. Cell apoptosis was then confirmed by morphological observations with annexin V and Hoechst 33342 fluorescence staining and by inhibition of the apoptotic pathway.

The cells examined in this study were obtained from inflamed synoviums of dogs affected by spontaneous stifle OA. Synovitis in OA is thought to develop secondary to noninflammatory arthropathy, and its extent would be relatively mild when compared with rheumatoid arthritis (RA). However, in many cases of OA, synovitis has a negative impact on physical condition and leads to severe degeneration in affected joints. In some cases with severe OA, the extent of inflammation sometimes resembles inflammatory disorders of RA [9, 13].

In this study, tepoxalin showed definite cytotoxic effects on CFLSs. Carprofen and meloxicam had no significant effects on cell viabilities at any examined doses. Inhibition of 5-LOX with a specific 5-LOX inhibitor, AA-861, showed clear cytotoxic effects on CFLSs. Morphological findings and a caspase-3 inhibition assay then showed that these cytotoxic effects of tepoxalin and AA-861 were induced as the results of inducing cell apoptosis. The results indicated that LOX inhibition may suppress synovium growth and that COX-2 selective inhibitors, widely used as anti-inflammatory drugs, would not affect the viabilities of inflammatory synoviocytes. This hypothesis was also supported by our results showing the inhibitory effects of 5-LOX metabolite on the cytotoxicity of tepoxalin and AA-861 and that 5-LOX activity could regulate survival of CFLSs. Tepoxalin, a COX/LOX dual inhibitor, would have unique inflammatory properties as an NSAIDs and directly or indirectly suppress viability of inflammatory synoviocytes by inhibition of LOX. Many reports have shown that 5-LOX plays an important role in various diseases associated with cell proliferation and that inhibition of 5-LOX resulted in cytotoxicity [10, 16, 31]. Overexpression of 5-LOX in tissue samples of primary tumor, as well as in established cancer cell lines, has been reported [5]. Important roles of 5-LOX and its

products in the survival and growth of human tumor cells have also been reported [19, 30]. Tepoxalin might somehow support suppression of LOX-dependent cell growth.

In this study, a higher tepoxalin concentration was necessary to induce definite apoptosis than the physiological concentration at oral administration of the recommended dose. These findings could explain why the LOX inhibitory effects of tepoxalin may suppress LOX-dependent cell growth *in vitro* but are not strong enough *in vivo*. A recent report showed that the 5-LOX metabolite, leukotriene B_4 (LTB_4) promotes migratory and invasive behavior and induces inflammation to fibroblast-like-synoviocytes of mice [6]. It is known that tepoxalin inhibits LTB_4 *in vivo* at a low concentration [2]. These reports suggest the advantageous effects of 5-LOX inhibition in addition to COX inhibition. Lipoxin is an eicosanoid produced by LOX catalysis of arachidonic acid. These LOX products play roles as physiological mediators to optimize articular cartilage metabolism [8]. Thus, complete inhibition of LOX could lead to severe adverse effects. It is known that tepoxalin inhibits less than half of the lipoxygenase activity, and production of 5-LOX metabolites is not completely blocked at recommended concentrations *in vivo* [2]. Mild inhibition of LOX by tepoxalin might be more favorable to use than specific LOX inhibitors.

In the present study, all data were obtained by *in vitro* study. This experimental condition did not always represent actual synovium conditions in OA. The number of cell sources was also not satisfactory enough to prove the effects of tepoxalin on the OA synovium *in vivo*. However, regarding its anti-inflammatory properties, it appears that tepoxalin had a significant proapoptotic effect on CFLSs at relatively high concentrations [7]. Further studies are required to determine more accurate clinical efficacy for practical use of tepoxalin.

In conclusion, tepoxalin appeared to reduce the number of proliferating synovial cells *in vitro*, possibly by inhibiting LOX and direct effects. These findings suggest that the clinical effects of tepoxalin could result not only from alleviation of pain but also from direct antiproliferative effects on the inflamed synovium.

REFERENCES

1. Agnello, K. A., Reynolds, L. R. and Budsberg, S. C. 2005. In vivo effects of tepoxalin, an inhibitor of cyclooxygenase and lipoxygenase, on prostanoid and leukotriene production in dogs with chronic osteoarthritis. *Am. J. Vet. Res.* **66**: 966–972. [Medline] [CrossRef]
2. Argentieri, D. C., Ritchie, D. M., Ferro, M. P., Kirchner, T., Wachter, M. P., Anderson, D. W., Rosenthale, M. E. and Capetola, R. J. 1994. Tepoxalin: a dual cyclooxygenase/5-lipoxygenase inhibitor of arachidonic acid metabolism with potent anti-inflammatory activity and a favorable gastrointestinal profile. *J. Pharmacol. Exp. Ther.* **271**: 1399–1408. [Medline]
3. Baier, A., Meineckel, I., Gay, S. and Pap, T. 2003. Apoptosis in rheumatoid arthritis. *Curr. Opin. Rheumatol.* **15**: 274–279. [Medline] [CrossRef]
4. Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D. and Mitchell, J. B. 1987. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assesment of chemosensitivity testing. *Cancer Res.* **47**: 936–942. [Medline]
5. Chen, M., Lam, B. K., Luster, A. D., Zarini, S., Murphy, R. C., Bair, A. M., Soberman, R. J. and Lee, D. M. 2010. Joint tissues amplify inflammation and alter their invasive behavior via leukotriene B4 in experimental inflammatory arthritis. *J. Immunol.* **185**: 5503–5511. [Medline] [CrossRef]
6. Chen, X., Sood, S., Yang, C. S., Li, N. and Sun, Z. 2006. Five-lipoxygenase pathway of arachidonic acid metabolism in carcino-genesis and cancer chemoprevention. *Curr. Cancer Drug Targets* **6**: 613–622. [Medline] [CrossRef]
7. Clark, T. P. 2006. The Clinical pharmacology of cyclooxygenase-2-selective and dual inhibitors. *Vet. Clin. North Am. Small Anim. Pract.* **36**: 1061–1085. [Medline] [CrossRef]
8. Conte, F. P., Menezes-de-Lima, O. Jr., Verri, W. A. Jr., Cunha, F. Q., Penido, C. and Henriques, M. G. 2010. Lipoxin A(4) attenutates zymosan-induced arthritis by modulating endothelin-1 and its effects. *Br. J. Pharmacol.* **161**: 911–924. [Medline] [CrossRef]
9. Farahat, M. N., Yanni, G., Poston, R. and Panayi, G. S. 1993. Cytokine expression in synovial membranes of patients with rheumatoid arthritis and osteoarthritis. *Ann. Rheum. Dis.* **52**: 870–875. [Medline] [CrossRef]
10. Gheorghe, K. R., Korotkova, M., Catrina, A. I., Backman, L., af Klint, E., Claesson, H. E., Rådmark, O. and Jakobsson, P. J. 2009. Expression of 5-lipoxygenase and 15-lipoxygenase in rheumatoid arthritis synovium and effects of intraarticular glucocorticoids. *Arthritis Res. Ther.* **11**: R83. [Medline] [CrossRef]
11. Gilmour, M. A. and Lehenbauer, T. W. 2009. Comparison of tepoxalin, carprofen, and meloxicam for reducing intraocular inflammation in dogs. *Am. J. Vet. Res.* **70**: 902–907. [Medline] [CrossRef]
12. Goodman, L., Coles, T. B. and Budsberg, S. 2008. Leukotriene inhibition in small animal medicine. *J. Vet. Pharmacol. Ther.* **31**: 387–398. [Medline] [CrossRef]
13. Haraoui, B., Pelletier, J. P., Cloutier, J. M., Faure, M. P. and Martel-Pelletier, J. 1991. Synovial membrane histology and immunopathology in rheumatoid arthritis and osteoarthritis. In vivo effects of antirheumatic drugs. *Arthritis Rheum.* **34**: 153–163. [Medline] [CrossRef]
14. Johnston, S. A. 1997. Osteoarthristis. Joint anatomy, physiology, and pathobiology. *Vet. Clin. North Am. Small Anim. Pract.* **27**: 699–723. [Medline]
15. Kapoor, M., Martel-Pelletier, J., Lajeunesse, D., Pelletier, J. P. and Fahmi, H. 2011. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat. Rev. Rheumatol.* **7**: 33–42. [Medline] [CrossRef]
16. Kuhn, H., Chaitidis, P., Roffeis, J. and Walther, M. 2007. Arachidonic acid metabolites in the cardiovascular system: the role of lipoxygenase isoforms in atherogenesis with particular emphasis on vascular remodeling. *J. Cardiovasc. Pharmacol.* **50**: 609–620. [Medline] [CrossRef]
17. Kusunoki, N., Yamazaki, R. and Kawai, S. 2002. Induction of apoptosis in rheumatoid synovial fibroblasts by celecoxib, but not by other selective cyclooxygenase 2 inhibitors. *Arthritis Rheum.* **46**: 3159–3167. [Medline] [CrossRef]
18. Laufer, S. 2004. Osteoarthritis therapy—are there still unmet needs? *Rheumatology* **43**: i9–i15 (oxford). [Medline] [CrossRef]
19. Maccarone, M., Melino, G. and Finazzi-Agrò, A. 2001. Lipoxygenase and their involvement in programmed cell death. *Cell Death Differ.* **8**: 776–784. [Medline] [CrossRef]

20. Macrory, L., Vaughan-Thomas, A., Clegg, P. D. and Innes, J. F. 2009. An exploration of the ability of tepoxalin to ameliorate the degradation of articular cartilage in a canine *in vitro* model. *BMC Vet. Res.* **5**: 25. [Medline] [CrossRef]

21. Pearle, A. D., Scanzello, C. R., George, S., Mandl, L. A., DiCarlo, E. F., Peterson, M., Sculco, T. P. and Crow, M. K. 2007. Elevated high-sensitivity C-reactive protein levels are associated with local inflammatory findings in patients with osteoarthritis. *Osteoarthritis Cartilage* **15**: 516–523. [Medline] [CrossRef]

22. Pelletier, J. P., Martel-Pelletier, J. and Abramson, S. B. 2001. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. *Arthritis Rheum.* **44**: 1237–1247. [Medline] [CrossRef]

23. Punke, J. P., Speas, A. L., Reynolds, L. R. and Budsberg, S. C. 2008. Effects of firocoxib, meloxicam, and tepoxalin on prostanoid and leukotriene production by duodenal mucosa and other tissues of osteoarthritic dogs. *Am. J. Vet. Res.* **69**: 1203–1209. [Medline] [CrossRef]

24. Renberg, W. C. 2005. Pathophysiology and management of arthritis. *Vet. Clin. North Am. Small Anim. Pract.* **35**: 1073–1091. [Medline] [CrossRef]

25. Ritchlin, C. 2000. Fibroblast biology. Effector signals released by the synovial fibroblast in arthritis. *Arthritis Res.* **2**: 356–360. [Medline] [CrossRef]

26. Sanderson, R. O., Beata, C., Flipo, R. M., Genevois, J. P., Macias, C., Tacke, S., Vezzoni, A. and Innes, J. F. 2009. Systematic review of the management of canine osteoarthritis. *Vet. Rec.* **164**: 418–424. [Medline] [CrossRef]

27. Smith, M. D., Triantafillou, S., Parker, A., Youssef, P. P. and Coleman, M. 1997. Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. *J. Rheumatol.* **24**: 365–371. [Medline]

28. Sutton, S., Clutterbuck, A., Harris, P., Gent, T., Freeman, S., Foster, N., Barrett-Jolley, R. and Mobasheri, A. 2009. The contribution of the synovium, synovial derived inflammatory cytokines and neuropeptides to the pathogenesis of osteoarthritis. *Vet. J.* **179**: 10–24. [Medline] [CrossRef]

29. Vane, J. R. and Botting, R. M. 1996. Mechanism of action of anti-inflammatory drugs. *Scand. J. Rheumatol. Suppl.* **102**: 9–21. [Medline] [CrossRef]

30. Wagenknecht, B., Gulbins, E., Lang, F., Dichgans, J. and Weller, M. 1997. Lipoxygenase inhibitors block CD95 ligand-mediated apoptosis of human malignant glioma cells. *FEBS Lett.* **409**: 17–23. [Medline] [CrossRef]

31. Zhou, G. X., Ding, X. L., Huang, J. F., Zhang, H. and Wu, S. B. 2007. Suppression of 5-lipoxygenase gene is involved in triptolide-induced apoptosis in pancreatic tumor cell lines. *Biochim. Biophys. Acta* **1770**: 1021–1027. [Medline] [CrossRef]