In vivo effects of tepoxalin, an inhibitor of cyclooxygenase and lipoxygenase, on prostanoid and leukotriene production in dogs with chronic osteoarthritis

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Objective—To evaluate in vivo effects of tepoxalin, an inhibitor of cyclooxygenase (COX) and lipoxygenase (LOX), on prostaglandin (PG) and leukotriene production in osteoarthritic dogs.

Animals—7 mixed-breed adult dogs with chronic unilateral arthritis of a stifle joint.

Procedure—Dogs were treated in accordance with a randomized 3-way crossover design. Each dog received an inert substance, meloxicam, or tepoxalin for 10 days. On day 0 (baseline), 3, and 10, dogs were anesthetized and samples of blood, stifle joint synovial fluid, and gastric mucosa were collected. Concentrations of PGE_2 were measured in synovial fluid and after lipopolysaccharide stimulation of whole blood; PGE_1 and PGE_2 synthesis was measured in gastric mucosa. Thromboxane B_2 (TxB_2) concentration was measured in whole blood. Leukotriene B_4 (LTB_4) concentration was determined in gastric mucosa and in whole blood after ex vivo stimulation with a calcium ionophore.

Results—Tepoxalin significantly decreased LTB_4 concentrations in the blood and gastric mucosa at day 10 and TxB_2 concentrations in the blood and PGE_2 in the gastric mucosa and synovial fluid at days 3 and 10, compared with baseline values. Meloxicam significantly decreased PGE_2 concentrations in the blood at days 3 and 10 and synovial fluid at day 3. Meloxicam also decreased PGE_1 and PGE_2 synthesis in the gastric mucosa at day 3. Meloxicam did not affect LTB_4 synthesis in the blood or LTB_4 concentrations in the gastric mucosa.

Conclusions and Clinical Relevance—Tepoxalin has in vivo inhibitory activity against COX-1, COX-2, and 5-LOX in dogs at the current approved recommended dosage. (*Am J Vet Res* 2005;66:966–972)

Dual inhibitors of the arachidonic acid pathway that can alter the **cyclooxygenase (COX)** and **lipoxygenase (LOX)** pathways are beginning to be used in clinical veterinary practice. These dual inhibitors offer a potentially broader spectrum of inhibition of inflammatory mediators, and it has been proposed that they have fewer adverse effects on the gastrointestinal tract than currently used **nonsteroidal anti-inflammatory drugs (NSAIDs)**.[1-3]

The roles of the COX enzymes are complex. The COX-1 isoform is believed to be mainly a constitutively active enzyme responsible for homeostatic functions in tissues. Conversely, COX-2, the inducible isoform, is mostly activated in response to proinflammatory stimuli. The idea that inhibition of COX-1 results in the undesirable adverse effects associated with NSAIDs led to the development of COX-1–sparing drugs. The use of these COX-1–sparing NSAIDs has resulted in fewer toxic events but unfortunately has not completely eliminated the adverse effects.[4-6] The original hypothesis that these 2 COX isoforms had exclusive functions appears to be a generalization, and overlap in their functions exists. For example, COX-1 can be upregulated at sites of active inflammation.[3,7] It has been suggested that COX-2 can be found in normal gastric mucosa[8] and is important for healing of gastric ulcers.[9,10]

Gastrointestinal toxicosis is one of the most common adverse effects seen with NSAID use in dogs.[11] The inhibition of **prostaglandin (PG)** synthesis in the gastrointestinal mucosa can increase the susceptibility to injury[12,13]; however, it again appears that inhibition of COX-1 is not the sole contributor to NSAID-induced damage. Neutrophils appear to be activated and attracted to the gastrointestinal tract during treatment with NSAIDs.[14] One of the mediators of granulocyte activation is **leukotriene B_4 (LTB_4)**,[15,16] and increased synthesis of gastric LTB_4 has been reported during NSAID treatment.[17] One proposed cause of this increase in gastric LTB_4 production is that inhibition of 1 or both of the COX enzymes results in an abundance of substrates that subsequently may be shunted into other arachidonic acid metabolic pathways, such as the 5-LOX pathway, which ultimately results in an increase in LTB_4 production (**Figure 1**). This increased concentration of LTB_4 subsequently stimulates chemotaxis, adhesion, and degranulation of neutrophils, which results in mucosal damage.[18] Furthermore, it has been clearly documented[2,19] that NSAIDs increase neutrophil adhesion and migration in the mesenteric venules, a phenomenon not seen with tepoxalin. In fact, tepoxalin can protect against indomethacin-induced increases in neutrophil adhesion.[2,19] Analysis of these findings suggests that LOX inhibition may be beneficial in protecting the gastrointestinal mucosa from damage attributable to COX inhibition. Also, because the products of the LOX pathway play an important role in mediating inflammation,[20] inhibition of this pathway may

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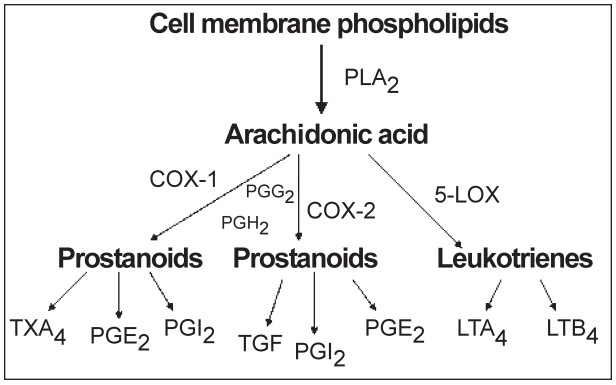

Figure 1—Schematic depicting arachidonic acid metabolism and functions of the cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. PLA_2 = Phospholipase A_2. PGG_2 = Prostaglandin G_2. PGH_2 = Prostaglandin H_2. TXA_4 = Thromboxane A_4. PGE_2 = Prostaglandin E_2. PGI_2 = Prostaglandin I_2. TGF = Transforming growth factor. LTA_4 = Leukotriene A_4. LTB_4 = Leukotriene B_4.

offer another method for blockade of the inflammatory cascade.

Tepoxalin, a dual inhibitor of COX and LOX, is approved for use in dogs. This compound can inhibit both COX and 5-LOX, has potent anti-inflammatory activity, and has a favorable pattern of effects on the gastrointestinal tract.[1,2] However, the in vivo ability of tepoxalin to inhibit both COX and LOX activity has not been investigated in dogs with **osteoarthritis (OA)**. Therefore, the objective of the study reported here was to examine in vivo effects of tepoxalin on PG and leukotriene production in several target tissues, including whole blood, gastric mucosa, and synovial fluid, of dogs with OA. Our hypothesis was that tepoxalin administered at approved recommended dosages would inhibit both the COX and LOX pathways of arachidonic acid metabolism.

Materials and Methods

Animals—Seven adult male mixed-breed hound-type dogs that weighed between 25 and 40 kg and had unilateral OA of 1 stifle joint were used in the study. The dogs were part of a research colony at the University of Georgia. Osteoarthritis was created by surgically induced injury to the cranial cruciate ligament several years prior to the study reported here. The contralateral nontransected stifle was considered the unaffected stifle joint. The study was reviewed and approved by the University of Georgia Animal Care and Use Committee.

Except for the OA, all dogs were considered to be in good health with expected findings for physical examination and laboratory results that were within the reference range for CBC count, serum biochemical analysis, and urinalysis performed immediately prior to commencement of the study. Gastric biopsy specimens were endoscopically obtained and evaluated for inflammation and colonization with *Helicobacter* spp. The dogs received no medication during the study, except for monthly heartworm preventative. Furthermore, the dogs were not receiving medical treatment for the OA prior to inclusion in this study.

Experimental design—Dogs were treated in accordance with a randomized 3-way crossover design. Each dog received an inert substance (no treatment), meloxicam[a] (0.2 mg/kg, PO, for the initial loading dose; then 0.1 mg/kg, PO, q 24 h), or tepoxalin[b] (20 mg/kg, PO, for the initial loading dose; then 10 mg/kg, PO, q 24 h) for 10 days. There was at least a 3-week interval between successive treatments. All drugs were obtained through the teaching hospital pharmacy. Meloxicam was chosen as a positive control treatment because of its COX-1–sparing activity.[21] The first day of treatment was designated day 0 (baseline); doses were administered after sample collection.

On days 0, 3, and 10, samples of blood, synovial fluid from the stifle joints, and gastric biopsy specimens were collected. After collection of blood samples by use of venipuncture, each dog was anesthetized by use of propofol[c] (4 mg/kg) and maintained on halothane.[d] Synovial fluid was collected via arthrocentesis from both stifle joints. Additionally, gastroscopy was performed and endoscopic biopsy specimens collected from the gastric antrum near the pylorus. The investigators were unaware of the treatment administered to each dog, and the same investigator performed the collection (KAA) and processing (LRR) of the samples to lessen variability.

Measurement of thromboxane B_2 in whole blood—Six milliliters of blood was collected via venipuncture into an evacuated siliconized glass tube, immediately placed into a water bath (37°C), and incubated for 1 hour. Indomethacin[e] (final concentration, 30µM) was subsequently added to stop further synthesis of thromboxane, and **thromboxane B_2 (TxB_2)** was measured as described elsewhere.[21]

Measurement of PGE_2 in whole blood—Four milliliters of blood was collected by venipuncture into heparinized tubes, and 500 µL of heparinized whole blood was placed in a microcentrifuge tube. Bacterial **lipopolysaccharide (LPS**; *Escherichia coli* serotype 127:B8)[f] was added to each tube to stimulate PGE_2 production, and PGE_2 concentrations were measured as described elsewhere.[21]

Measurement of LTB_4 in whole blood—Blood was collected into heparinized tubes. One milliliter of heparinized whole blood was aliquoted into microcentrifuge tubes and challenged with calcium ionophore A23187[g] to stimulate LTB_4 production as described in another study.[1]

Measurement of PGE_2 in synovial fluid—Synovial fluid was collected from the affected and unaffected stifle joints via use of a standard arthrocentesis technique; samples were placed in microcentrifuge tubes. The PGE_2 concentration was measured as described elsewhere.[21]

Measurement of PG synthesis in gastric mucosa—All endoscopically obtained gastric biopsy specimens were processed within 8 minutes after collection. Specimens weighing < 4 mg were discarded. Synthesis of PGE_1 and PGE_2 was stimulated via mincing as described elsewhere.[21]

Measurement of LTB_4 synthesis in gastric mucosa—Biopsy specimens were flash-frozen in liquid nitrogen and then stored at –70°C until assayed. At the time of the assay, each biopsy specimen was thawed, weighed, and placed in 1 mL of Tris buffer. Biopsy specimens were then finely minced (15 seconds), vortexed for 3 minutes, and centrifuged to yield a pellet. The supernatant was removed, and LTB_4 concentrations were measured by use of an ELISA.[h]

Statistical analysis—A repeated-measures ANOVA was used to compare data within and between treatments. When significant ($P < 0.05$) changes were detected, means of interest were compared by use of a Tukey post hoc analysis.

Results

Animals—Six of 7 dogs had some colonization with *Helicobacter* spp, but none of the dogs had sub-

stantial inflammation or clinical signs associated with gastrointestinal tract disease. Because of the high prevalence of clinically normal dogs with colonization by *Helicobacter* spp, all dogs were included in the study. One dog died suddenly at day 9 during the third treatment period while receiving meloxicam. Necropsy was performed, and a focal, peracute perforating duodenal ulcer was identified.

Concentration of TxB_2 in whole blood— Tepoxalin significantly decreased TxB_2 concentrations over time, with significant decreases at days 3 and 10, compared with the baseline concentration (**Figure 2**). No changes in TxB_2 production were observed over time when dogs were administered the inert substance or meloxicam. A significant decrease in TxB_2 concentrations was seen at days 3 and 10 when dogs were administered tepoxalin, compared with TxB_2 concentrations when dogs were administered the inert substance or meloxicam.

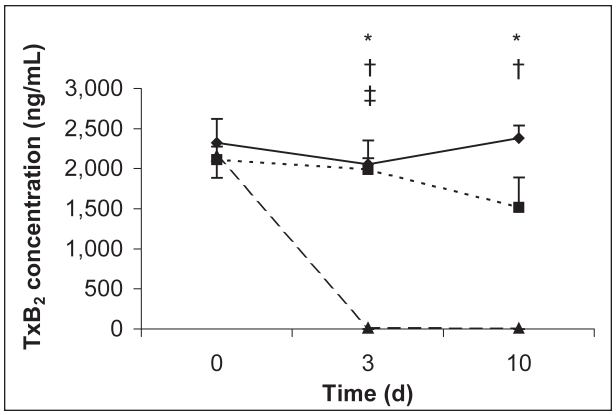

Figure 2—Mean ± SD concentrations of thromboxane B_2 (TxB_2) in whole blood of dogs during a 10-day period of oral administration of an inert substance (diamond), meloxicam (square), or tepoxalin (triangle). There was a minimum interval of at least 3 weeks between successive treatments. *Within the tepoxalin treatment, value differs significantly ($P < 0.05$) from the value for day 0. †Within a time period, value differs significantly ($P < 0.05$) between the tepoxalin treatment and the inert substance treatment. ‡Within a time period, value differs significantly ($P < 0.05$) between the tepoxalin treatment and the meloxicam treatment. Day 0 = First day of administration.

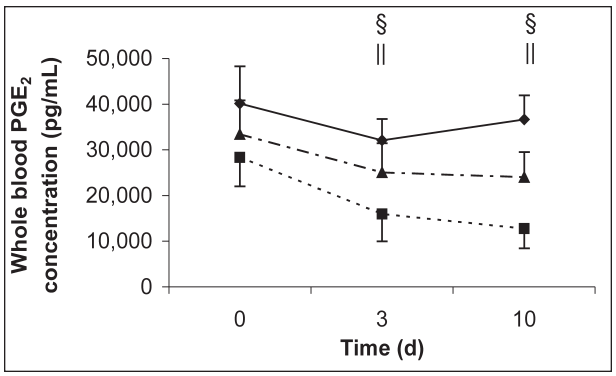

Figure 3—Mean ± SD concentrations of prostaglandin (PG)E_2 in lipopolysaccharide-stimulated whole blood of dogs during a 10-day period of oral administration of an inert substance (diamond), meloxicam (square), or tepoxalin (triangle). There was a minimum interval of at least 3 weeks between successive treatments. §Within the meloxicam treatment, value differs significantly ($P < 0.05$) from the value for day 0. ‖Within a time period, value differs significantly ($P < 0.05$) between the meloxicam treatment and the inert substance treatment. Day 0 = First day of administration.

Concentration of PGE_2 in whole blood— Meloxicam significantly decreased PGE_2 concentrations at days 3 and 10, compared with the baseline concentration (**Figure 3**). A significant decrease in PGE_2 concentration was seen at day 10 when dogs were administered meloxicam, compared with the concentration when dogs were administered the inert substance. Tepoxalin did not significantly alter PGE_2 production from WBCs that were stimulated by LPS.

Concentration of LTB_4 in whole blood— Concentration of LTB_4 was significantly decreased at day 10, compared with the baseline concentration, when dogs were administered tepoxalin. We also detected a significant decrease in LTB_4 synthesis at day 10 when dogs were administered the inert substance. There was a significantly lower concentration of LTB_4

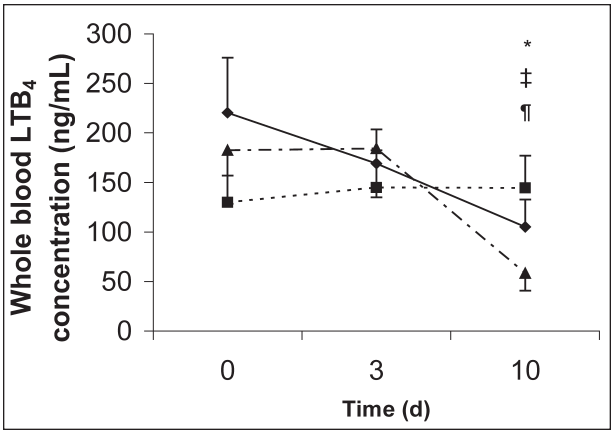

Figure 4—Mean ± SD concentrations of leukotriene B_4 (LTB_4) in calcium ionophore–stimulated whole blood of dogs during a 10-day period of oral administration of an inert substance (diamond), meloxicam (square), or tepoxalin (triangle). There was a minimum interval of at least 3 weeks between successive treatments. ¶Within the inert substance treatment, value differs significantly ($P < 0.05$) from the value for day 0. *See* Figure 2 for remainder of key.

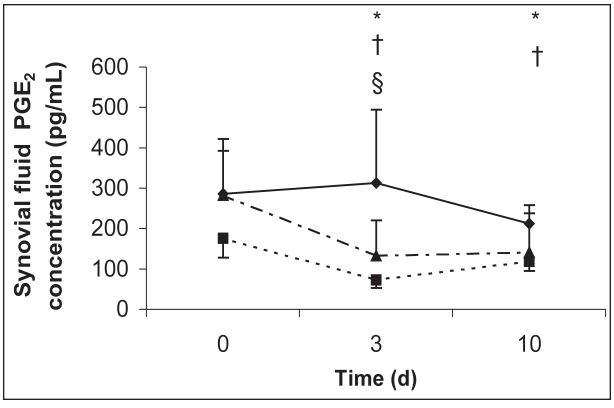

Figure 5—Mean ± SD concentrations of PGE_2 in synovial fluid obtained from the osteoarthritis-affected stifle joint of dogs during a 10-day period of oral administration of an inert substance (diamond), meloxicam (square), or tepoxalin (triangle). There was a minimum interval of at least 3 weeks between successive treatments. *See* Figures 2 and 3 for remainder of key.

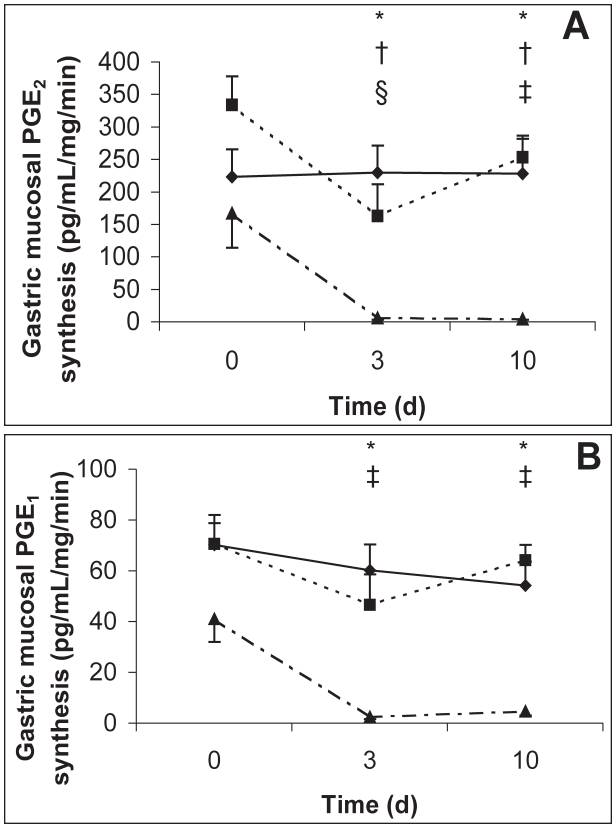

Figure 6—Mean ± SD synthesis of PGE_2 (A) and PGE_1 (B) in gastric mucosa obtained from dogs during a 10-day period of oral administration of an inert substance (diamond), meloxicam (square), or tepoxalin (triangle). There was a minimum interval of at least 3 weeks between successive treatments. *See* Figures 2 and 3 for remainder of key.

at day 10 when dogs were administered tepoxalin, compared with the concentration when they were administered meloxicam (**Figure 4**).

Concentration of PGE_2 in synovial fluid—When dogs were administered tepoxalin, PGE_2 concentrations were significantly increased at days 3 and 10 in synovial fluid collected from the affected stifle joint and at day 10 in synovial fluid collected from the unaffected stifle joint, compared with the baseline concentrations (**Figure 5**). When the dogs were administered meloxicam, there was a significant decrease in PGE_2 at day 3 in both the affected and unaffected stifle joints, compared with the baseline concentrations. A nonsignificant decrease was also detected at day 10 in the affected (P = 0.08) and unaffected (P = 0.07) stifle joints. There was a significant decrease in the PGE_2 concentration at days 3 and 10 for the affected stifle joint when dogs were administered tepoxalin, compared with the concentration for dogs when administered the inert substance; however, no significant differences were seen in the unaffected stifle joint when dogs were administered the various treatments. There was a nonsignificant decrease in PGE_2 concentration of synovial fluid collected from the affected stifle joint at days 3 (P = 0.052) and 10 (P = 0.08), compared with concentrations when dogs were administered the inert substance.

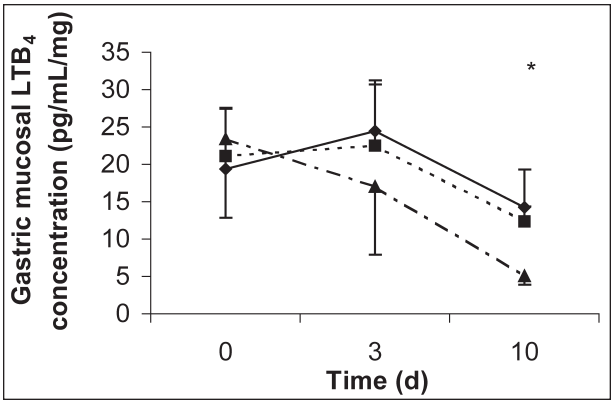

Figure 7—Mean ± SD concentrations of LTB_4 in gastric mucosa obtained from dogs during a 10-day period of oral administration of an inert substance (diamond), meloxicam (square), or tepoxalin (triangle). There was a minimum interval of at least 3 weeks between successive treatments. *See* Figure 2 for key.

Synthesis of PGs in gastric mucosa—A significant decrease in PGE_2 synthesis in gastric mucosa was observed at days 3 and 10 when dogs were administered tepoxalin and at day 3 when dogs were administered meloxicam, compared with baseline concentrations (**Figure 6**). There was a significant decrease in PGE_2 synthesis at days 3 and 10 when dogs were administered tepoxalin, compared with concentrations when dogs were administered the inert substance. Synthesis of PGE_2 at day 10 when dogs were administered tepoxalin was also significantly decreased from the synthesis when the dogs were administered meloxicam. No significant differences in synthesis of PGE_2 in gastric mucosa were evident between the periods when dogs were administered meloxicam or the inert substance.

When dogs were administered tepoxalin, synthesis of PGE_1 in gastric mucosa was significantly decreased at days 3 and 10, compared with the baseline concentration (Figure 6). Synthesis of PGE_1 at days 3 and 10 was significantly less when dogs were administered tepoxalin, compared with when dogs were administered the inert substance or meloxicam. No significant differences in synthesis of PGE_1 in gastric mucosa were evident between the periods when dogs were administered meloxicam or the inert substance.

Concentration of LTB_4 in gastric mucosa—When dogs were administered tepoxalin, there was a significant decrease in LTB_4 concentrations in the gastric mucosa at day 10, compared with the baseline value. The concentration of LTB_4 when dogs were administered tepoxalin was lower at day 10, but not significantly so, compared with the concentrations when dogs were administered the inert substance or meloxicam (**Figure 7**). No significant differences in LTB_4 concentrations in gastric mucosa were evident during the period when dogs were administered meloxicam or the inert substance or between the periods when dogs were administered meloxicam or the inert substance.

Discussion

Traditionally, selectivity of NSAIDs has been assessed by in vitro methods. However, because of

the limitations of in vitro testing to determine clinically relevant NSAID selectivity,[22] an in vivo technique was developed in dogs.[21] In the study reported here, we used this technique to determine the in vivo COX activity of the NSAID tepoxalin in dogs. Meloxicam was chosen as a positive control drug because of its in vitro[23-26] and in vivo COX-1–sparing activity.[21]

The production of TxB_2 by platelets and synthesis of PGE_1 and PGE_2 in normal gastric mucosa are a function of COX-1 and therefore a reflection of COX-1 activity in the body.[27-29] Results from the study reported here revealed that tepoxalin suppressed TxB_2 production, as well as PGE_1 and PGE_2 production, by the gastric mucosa after 3 days of treatment. This indicates that tepoxalin administered at the approved therapeutic dosage inhibits COX-1 activity in dogs. These findings are consistent with results from an in vitro study[1] in which investigators examined tepoxalin. Conversely, meloxicam was unable to affect TxB_2 production by platelets, supporting its COX-1–sparing classification.

Surprisingly, meloxicam significantly decreased PGE_2 production in the gastric mucosa at day 3 but not at day 10. This pattern was also observed in PGE_1 concentrations of the gastric mucosa, although significant differences were not achieved. In another study,[21] PG concentrations were measured at days 7 and 21 of treatment, and a decrease in PG production was not observed in the meloxicam-treated dogs at either time point. Most likely, an early decrease went undetected because of the longer interval before measurement of PGE_2. The current findings of a decrease at day 3 represent a loss of PGE_2 production attributable to inhibition of constitutive gastric mucosal COX-1 or COX-2. Given that PGE_1 was similarly affected, it could be speculated that the loss was more likely attributable to inhibition of COX-1. The return of PGE_1 and PGE_2 by day 10 may suggest an adaptation of the gastric mucosa to upregulate PGE_2 production via COX-1 in the gastric mucosa. Similar findings of decreased PGE_2 concentrations on day 3 during administration of deracoxib, carprofen, and etodolac have also been reported.[29] Analysis of these data suggests that meloxicam was able to spare COX-1 inhibition in platelets at all time periods and PG production in the gastric mucosa after 3 days of treatment.

The production of PGE_2 by LPS-stimulated WBCs is a function of COX-2.[30] Similarly, synthesis of PGE_2 by inflamed synovial tissue in humans mainly results from COX-2 induction.[31] Therefore, measurement of PGE_2 concentrations in synovial fluid of inflamed joints most likely represents COX-2 activity. Analysis of our results revealed that tepoxalin did not significantly alter PGE_2 production by LPS-stimulated WBCs; however, in the affected stifle joints, tepoxalin significantly decreased PGE_2 concentrations. Production of PGE_2 was also measured in synovial fluid obtained from the contralateral unaffected stifle joint; tepoxalin was also able to decrease the PGE_2 concentrations in the unaffected joints over time. Analysis of these results suggests that tepoxalin is able to inhibit COX-2 in dogs when administered at this dosage. However, the cause of the inability of tepoxalin to inhibit PGE_2 synthesis by WBCs is not known. It may simply be that, at the current dosage, concentrations of tepoxalin in whole blood were not sufficiently high to suppress PGE_2 production in ex vivo LPS-stimulated WBCs. If this finding can be confirmed by use of this particular assay method, it will provide additional evidence to suggest that researchers and clinicians must be careful in the interpretation of in vitro ratios between COX-1 and COX-2 for this agent and potentially other agents. A similar result was seen by use of this same method in dogs when examining the NSAID etodolac.[29] Analysis of these data emphasizes that caution must be used when in vitro assay results are interpreted by investigators, and perhaps > 1 in vitro system must be used to test any particular agent.

When dogs were administered meloxicam, PGE_2 synthesis by the LPS-stimulated WBCs was significantly decreased. Meloxicam decreased PGE_2 concentrations in synovial fluid obtained from both stifle joints, although significant decreases were not achieved at all time points. These results are consistent with those reported in another study[21] in which meloxicam inhibited COX-2 activity and had COX-1–sparing activity.

Concentrations of LTB_4 have been measured in numerous cell types and for various inflammatory diseases. They have been measured by use of ex vivo, in vitro, and in vivo techniques.[32-34] In the study reported here, we used an ex vivo method of stimulation of whole blood with a calcium ionophore and in vivo measurement of LTB_4 concentrations in the gastric mucosa as indicators of LOX activity. Analysis of our data for whole blood revealed that tepoxalin significantly decreased LTB_4 synthesis by the calcium-stimulated WBCs, which supports the claim that, at the approved dosage, tepoxalin is able to inhibit 5-LOX in dogs. Interestingly, when dogs were administered the inert substance, a significant decrease of LTB_4 synthesis was observed over time for the calcium-stimulated WBCs, whereas LTB_4 synthesis remained unchanged in the calcium-stimulated WBCs when dogs were administered meloxicam. The reason for this decrease when dogs were administered the inert substance is unknown at this time. The decreasing concentrations of LTB_4 by the gastric mucosa when tepoxalin was administered also support its ability to inhibit 5-LOX in dogs in vivo. Interestingly, in contrast to synthesis of LTB_4 by whole blood when dogs were administered tepoxalin, gastric concentrations of LTB_4 remained unchanged when dogs were administered meloxicam or the inert substance. Increases in concentrations of leukotrienes, including LTB_4, impair mucosal microcirculation through potent effects on leukocyte aggregation and vasoconstriction.[16,35] Impaired mucosal microcirculation (stasis) has been associated with NSAID-induced injury of the gastric mucosa,[36] and overproduction of 5-LOX products has been documented[17,37] in the gastric mucosa and gastric circulation following treatment with NSAIDs. The effect of leukotrienes on reduction of mucosal blood flow attributable to NSAID administration remains controversial,[36,38-40] but the data indirectly appear to be quite credible. The fact that meloxicam did not increase LTB_4 concentrations in a manner similar to that for other

NSAIDs is consistent with data reported in studies[41] conducted in mice. The exact mechanism for this difference has yet to be elucidated.[42] One possible explanation is that for the COX-1–sparing drugs, there is still arachidonic acid metabolism attributable to COX-1, and thus there is not the same amount of shunting of arachidonic acid into the LOX metabolic pathway; therefore, there is not an overproduction of 5-LOX products that has been reported[17,37] for nonspecific COX inhibitors.[17,37] Additional testing of other COX-1–sparing drugs will be necessary to explore this hypothesis.

In the study reported here, we determined that administration of tepoxalin at the current approved recommended dosage has inhibitory activity against COX-1 and COX-2 as well as 5-LOX in dogs. Thus, this is the first agent in this novel class of anti-inflammatory agents approved for veterinary use that will inhibit PGs and leukotrienes. Results of the positive control drug, meloxicam, were consistent with results of another in vivo study[21] in dogs, which confirms the classification as a COX-1–sparing NSAID.

a. Metcam, Boehringer Ingelheim, Vetmedica Incorporated, St Joseph, Mo.
b. Zubrin, Schering Plough Animal Health Corp, Union, NJ.
c. Propofol, Schering Plough Animal Health, Union City, NJ.
d. Halothane, Halocarbon Laboratories, River Edge, NJ.
e. Indomethacin, Cayman Chemical Co, Ann Arbor, Mich.
f. Lipopolysaccharide E. coli serotype 127:B8, Sigma Chemical Co, St Louis, Mo.
g. Calcium inonphore A23187, Sigma Chemical Co, St Louis, Mo.
h. Leukotriene B_4 EIA kit, Cayman Chemical Co, Ann Arbor, Mich.

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