In vivo effects of meloxicam and aspirin on blood, gastric mucosal, and synovial fluid prostanoid synthesis in dogs

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Objective—To evaluate in vivo activityin dogs of meloxicam or aspirin, previously shown in vitro to be a selective cyclooxygenase-2 (COX-2) inhibitor (COX-1 sparing drug), or a nonselective COX inhibitor, respectively.

Animals—12 male dogs with unilateral osteoarthritis of the stifle joint.

Procedure—Each dog was treated in a crossover design with aspirin or meloxicam for 21 days. Prostaglandin E_2 (PGE_2) concentrations were measured at days 0 (baseline), 7, and 21 of each treatment period in lipopolysaccharide (LPS)-stimulated blood, synovial fluid collected by arthrocentesis, and endoscopic gastric mucosal biopsy specimens. Thromboxane B_2 (TXB_2) was evaluated in blood on days 0, 7, and 21 of each treatment period.

Results—Aspirin administration significantly suppressed PGE_2 concentrations in blood, gastric mucosa, synovial fluid, and suppressed TXB_2 concentration in blood at days 7 and 21. Meloxicam administration significantly suppressed PGE_2 concentrations in blood and synovial fluid at days 7 and 21, but had no effect on concentrations of TXB_2 in blood or PGE_2 in gastric mucosa. Suppression of LPS-stimulated PGE_2 concentrations in blood and synovial fluid by aspirin and meloxicam administration is consistent with activity against the COX-2 isoenzyme. Suppression of concentrations of PGE_2 in the gastric mucosa and TXB_2 in blood by aspirin administration is consistent with activity against COX-1. Meloxicam, in contrast, had a minimal effect on functions mediated by COX-1.

Conclusions and Clinical Relevance—Meloxicam acts in vivo in dogs as a COX-1 sparing drug on target tissues by sparing gastric PGE_2 synthesis while retaining antiprostaglandin effects within inflamed joints. (*Am J Vet Res* 2002;63:1527–1531)

The use of **nonsteroidal anti-inflammatory drugs (NSAIDs)** for the management of pain in small animals is often limited by the toxicity of the drugs in these species. The therapeutic action and toxicity of NSAIDs are primarily thought to be the result of an inhibition of **cyclooxygenase (COX)** and subsequent prostaglandin formation.[1] The discovery of 2 separate isoforms of COX has revolutionized NSAID development. Cyclooxygenase-2, the inducible isoform, is believed to be primarily responsible for the inflammatory activity of prostaglandins. Cyclooxygenase-1, the constitutive isoform, is believed to be responsible for the basal physiologic functions, or overall maintenance, provided by prostaglandins. These actions include gastric mucosal protection, renal function, and platelet activity. Therefore, inhibition of COX-1 by NSAIDs is believed to result in most of the toxic effects of NSAIDs, and inhibition of COX-2 is believed to result in most of the therapeutic effects.[2] This theory has led to the development of presumably less toxic NSAIDs, which selectively inhibit COX-2 and spare COX-1 activity.

On the basis of this premise, NSAIDs are now being classified according to their COX selectivity. This selectivity is most often expressed as a ratio of the concentration at which a specific drug inhibits the activity of each isoenzyme by 50%. Many studies have evaluated the selectivity of NSAIDs by use of in vitro techniques. Among the various studies, most NSAIDs have a uniform trend in their COX selectivity. However, discrepancies among study results do exist, especially when evaluating the degree of COX selectivity for a particular agent.

A recent review of methods evaluating NSAIDs selectivity by use of in vitro techniques revealed differing methods that had variable results.[3] These in vitro assays may use recombinant enzymes or specific cell types known to have the desired COX expression. The type of cell used for COX expression, as well as the species of cell line, can differ. Some variation among studies may exist as a result of species differences in the expression and activity of the COX isoforms and, consequently, the activity of NSAID against them. For example, etodolac appears to be COX-1 sparing when substrate from human sources is used,[4,5] but COX-1 is selective when canine substrate is used.[6,7] Studies also vary in their methods, including the method of COX-2 induction and incubation times.

Another question regarding in vitro testing of NSAIDs selectivity is the in vivo relevance of these assays. For example, many NSAIDs are highly protein-bound in blood, thus affecting their distribution and in vivo activity. In addition, selectivity is dependent on drug concentration and could, therefore, be lost at high

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drug concentrations.[8] If NSAIDs are concentrated in tissue that is dependent on prostaglandins for function, such as the gastric mucosa, an increase in toxic effects could result. Therefore, in vitro selectivity of NSAIDs may not accurately predict the in vivo activity of that particular drug.

The objective of the study reported here was to evaluate the in vivo activity of 2 NSAIDs: meloxicam, a proposed COX-1 sparing NSAID, and aspirin, a nonselective NSAID, in dogs. We evaluated this activity against specific target tissues, the gastric mucosa and synovial fluid. In doing so, we hoped to further clarify the biologic actions of these drugs in vivo and provide a model for the future evaluation of NSAIDs selectivity.

Materials and Methods

Animals—Twelve adult male mixed-breed hound dogs weighing 25 to 40 kg, with unilateral osteoarthritis of the right stifle joint, were used in our study. The dogs are part of a research colony at the University of Georgia. Our study was reviewed and approved by the University of Georgia Animal Care and Use Committee. Osteoarthritis was caused by previously induced cranial cruciate ligament injury. Except for the osteoarthritis, all dogs were considered in good health with normal findings on physical examination and had laboratory results that were within reference range on CBC determination, serum biochemical analysis, and urinalysis. Gastric biopsy specimens were collected endoscopically prior to our study and evaluated for the presence of underlying inflammation and colonization with *Helicobacter* organisms.

Experimental design—Each dog received meloxicam[a] (0.2 mg/kg, PO, q 24 h) or aspirin (25 mg/kg, PO, q 12 h) for 21 days in a crossover design with a 6-month "washout" period between treatments. On days 0, 7, and 21 of each treatment period, blood was collected for evaluation of **thromboxane B_2 (TXB_2)** and **prostaglandin E_2 (PGE_2)**. In addition, each dog was anesthetized, induced with propofol[b] (4 mg/kg), and maintained on isoflurane.[c] Synovial fluid was collected from both stifle joints by a standard arthrocentesis technique. Gastroscopy was performed during each anesthetic episode, and 3 endoscopic biopsy specimens were collected from the gastric antrum.

Blood TXB_2 measurements—Six milliliters of blood were collected by venepuncture into an evacuated siliconized glass red-top tube, immediately placed in a 37°C water bath, and incubated for 1 hour. Indomethacin[d] was subsequently added to a final concentration of 30µM to stop further thromboxane synthesis. Tubes were centrifuged at room temperature (approx 18°C) for 10 minutes at 2,000 × *g*. Serum was transferred into 1 mL aliquots and frozen at –70°C pending analysis. All samples were analyzed together. The samples were thawed on ice, and a lipid extraction was performed by passage through an ethyl C_2 minicolumn.[e] Thromboxane B_2 was measured by use of an ELISA.[d]

Blood PGE_2 measurements—Four milliliters of blood were collected into heparinized green-top tubes, and 500 µL of the heparinized blood was placed in 3 microcentrifuge tubes. Fifty micrograms of bacterial **lipopolysaccharide (LPS**; *Escherichia coli* serotype 127:B8)[f] was added to each tube, and this combination was incubated at 37°C for 24 hours. After incubation, the tubes were centrifuged at 12,000 × *g* for 5 minutes. Plasma was mixed with 900 µL methanol and centrifuged again at 12,000 × *g* for 1 minute for extraction of prostaglandins. The sample was stored at –70°C until assayed for PGE_2 with an ELISA.[d]

Gastric mucosal PGE_2 synthesis—Pinch biopsy specimens of the gastric mucosa were collected endoscopically from the gastric antrum near the pylorus in each dog. To lessen variability, the same individuals throughout our study performed collection and processing of biopsy specimens. Also, all samples were processed within 8 minutes after removal from the stomach. Specimens weighing < 4 mg were discarded. Following removal, each biopsy specimen was placed in an individual microcentrifuge tube containing 1 mL of TRIS buffer (100mM, pH 7.8) and weighed. Biopsy specimen weight was determined after subtraction of this value from the prebiopsy specimen weight of the vial. Biopsy specimens were individually minced for 15 seconds with scissors and allowed to incubate in buffer for 3 minutes. They were briefly vortexed (3 to 5 seconds) and centrifuged for 15 seconds to pellet the tissue. The supernatant was extracted and replaced with 1 mL of fresh buffer. The sample with fresh buffer was vortexed for 3 minutes to generate prostaglandin synthesis. Next, the tube was centrifuged for 15 seconds and 900 µL of the supernatant was transferred to a tube containing 10 µg of indomethacin to stop further prostaglandin synthesis. The sample was allocated into 300 µL samples and stored at –80°C until PGE_2 was measured by use of an ELISA.[d] Results were reported as mg of PGE_2/kg/min.

Synovial fluid PGE_2—Synovial fluid was collected from stifle joints of each dog via a standard arthrocentesis technique. However, because of the limited and inconsistent amounts of fluid collected from the unaffected left stifle joint, an analysis was only performed on fluid from the right stifle joints. Fluid was immediately placed in a microcentrifuge tube. Fifty microliters of sample was mixed with 150 µL of citrate buffer (100mM, pH 3.0). Following extraction through a C18 minicolumn,[e] the PGE_2 was measured by use of an ELISA.[d]

Statistical analysis—A repeated measure ANOVA was used to compare PGE_2 and TXB_2 concentrations over time. If significant changes were found, means of interest were compared by use of a least-squared difference test. Significance was set at $P < 0.05$. Graphic results are expressed as percent change from baseline.

Results

No abnormalities were detected on the original physical examination, CBC determination, serum biochemical analysis, or urinalysis. One dog had a mass in the gastric antrum on endoscopic evaluation. Histologic evaluation of a biopsy specimen revealed that this mass was consistent with a benign gastric polyp, and the dog was kept in our study. Histologic evaluation of gastric biopsy specimens revealed mild lymphoplasmacytic inflammation in most dogs. Because dogs did not have any signs associated with gastrointestinal disease, and mild inflammation is often detected in clinically normal dogs, dogs were not excluded from our study on the basis of this finding. No dog had substantial inflammation. Also, all dogs had some degree of colonization with *Helicobacter* spp. However, because dogs had no clinical signs of gastrointestinal disease or minimal inflammation, and because *Helicobacter* organisms are a common finding in clinically normal dogs, dogs were not excluded from our study on the basis of this finding.

On day 7 of aspirin administration, a significant decreased blood TXB_2 concentration was found when compared with baseline (**Fig 1**). The significant suppression from baseline continued at day 21. Meloxicam

administration caused no significant changes in TXB_2 concentrations at 7 or 21 days when compared with baseline. When comparing between groups, significant differences were observed at 7 and 21 days, with lower TXB_2 concentrations in the aspirin group.

On day 7 of aspirin administration, a significant decrease in LPS-stimulated blood PGE_2 concentration was found when compared with baseline (**Fig 2**). However, after 21 days, the PGE_2 concentrations had significantly increased, compared with baseline. After 7 days of meloxicam administration, PGE_2 concentrations were significantly decreased, compared with baseline, and remained significantly lower at day 21. Comparison between groups revealed no difference at day 7, but at day 21 a significantly higher concentration of PGE_2 was found in the aspirin group.

On day 7 of aspirin administration, a significant decrease in gastric mucosal PGE_2 synthesis was found when compared with baseline and continued through day 21 (**Fig 3**). However, on day 7 of meloxicam administration, an increase in gastric mucosal PGE_2 synthesis was observed, compared with baseline, but this change

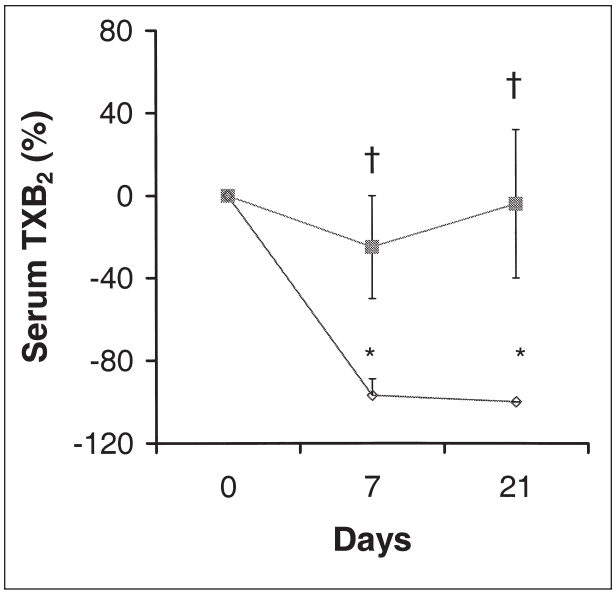

Figure 1—Mean ± SD percent change from baseline (day 0) of serum thromboxane B_2 (TXB_2) concentrations in dogs (n = 12) treated for 21 days with meloxicam (closed squares) or aspirin (open diamonds). *Significantly ($P < 0.05$) different from baseline values for each drug. †Significant ($P < 0.05$) difference between treatment groups at a given measurement point.

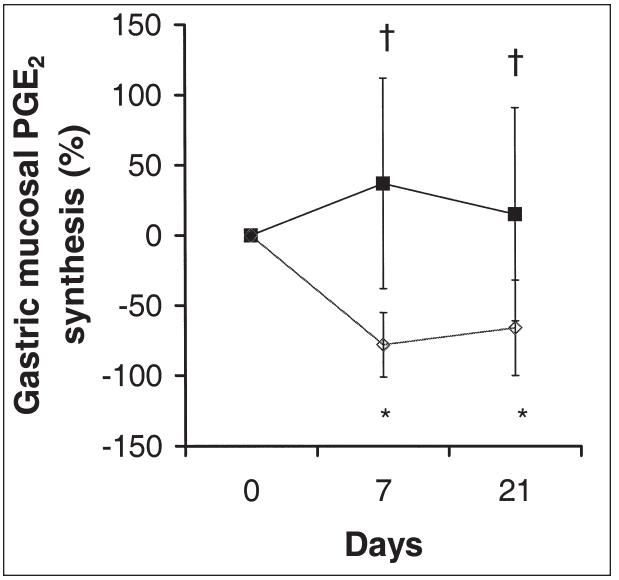

Figure 3—Mean ± SD percent change from baseline (day 0) of gastric mucosal PGE_2 synthesis in dogs (n = 12) treated for 21 days with meloxicam (closed squares) or aspirin (open diamonds). *See* Figure 1 for key.

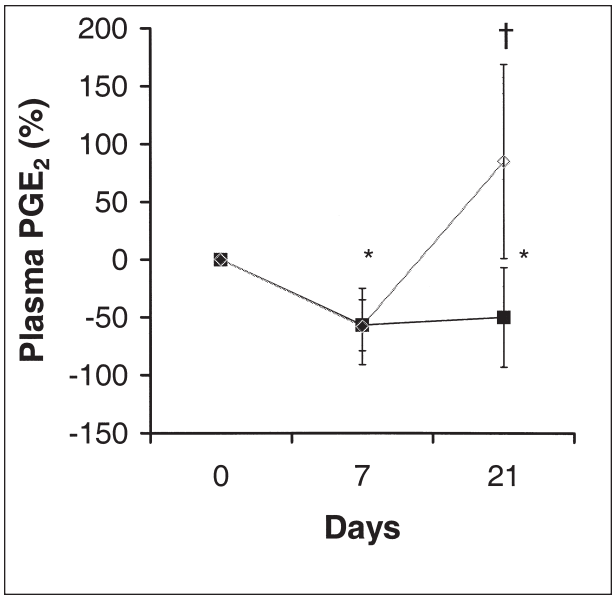

Figure 2—Mean ± SD percent change from baseline (day 0) of plasma prostaglandin E_2 (PGE_2) concentrations in dogs (n = 12) treated for 21 days with meloxicam (closed squares) or aspirin (open diamonds). *See* Figure 1 for key.

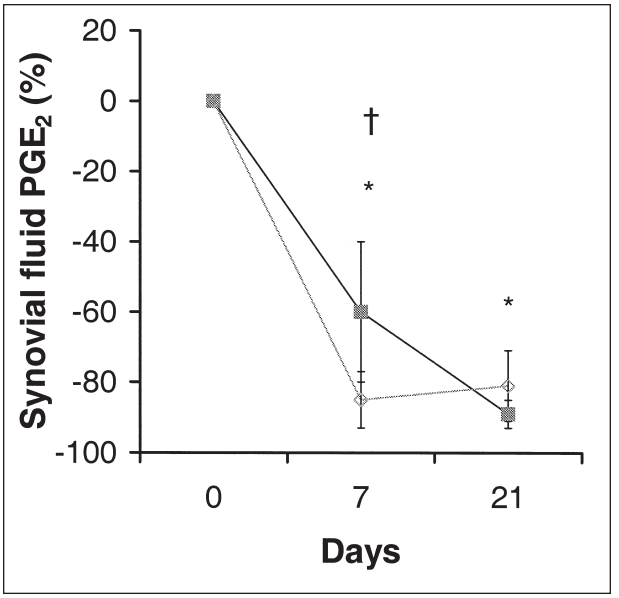

Figure 4—Mean ± SD percent change from baseline (day 0) of synovial fluid PGE_2 concentration in dogs (n = 12) treated for 21 days with meloxicam (closed squares) or aspirin (open diamonds). *See* Figure 1 for key.

was not significant. At day 21, the PGE_2 synthesis remained above baseline, but again this change was not significant. Comparison between groups revealed significantly higher PGE_2 synthesis in the meloxicam group at 7 and 21 days, compared with the aspirin group.

On day 7 of aspirin administration, PGE_2 concentration in synovial fluid was significantly decreased when compared with baseline and continued through day 21 (**Fig 4**). On day 7 of meloxicam administration, PGE_2 concentrations were also significantly decreased from baseline, and this decrease continued on day 21. Comparisons between groups revealed that aspirin caused a significantly greater suppression in PGE_2 concentrations at day 7 than did meloxicam, but by 21 days no differences were observed.

Discussion

The goal of our study was to evaluate the in vivo activity of 2 NSAIDs with known COX selectivity and to create an in vivo model for the future evaluation of NSAIDs selectivity. Aspirin was chosen because of its nonselective inhibition. The aspirin dose and frequency of administration are within the standard recommended therapeutic guidelines.[9] Meloxicam was chosen because of its purported COX-1 sparing effects, and the dose under study is considered a loading dose.[10] Meloxicam has an elimination half-life of approximately 24 hours, which allows for once-a-day dosing.[11] Aspirin and meloxicam have been shown to be non-COX selective and COX-1 sparing, respectively, in previous studies and most importantly in studies using canine origin cells.[6,7,12,13] In addition, the drugs have been shown to have gross effects on the gastric mucosa consistent with their selectivity.[14,15] The effects of 2 NSAIDs on 2 target areas, the gastric mucosa and synovial fluid, were evaluated by measuring prostaglandin production before and after administration of each drug at commonly prescribed doses. Production of thromboxane by platelets and LPS-induced PGE_2 in blood after administration of these drugs was also monitored. Incubation of heparinized blood with bacterial LPS for 24 hours results in a time-dependent enhancement of PGE_2 from monocytes.[16] To strengthen the power of our study, a crossover design was used to allow for a small group of dogs to be evaluated.

Prostaglandin production in the grossly normal stomach has been shown to be a function of COX-1.[17] Likewise, platelets produce COX-1, making thromboxane production a function of this isoform.[5,18] Therefore, measurement of prostaglandin and thromboxane production by gastric mucosa and platelets, respectively, should correlate to COX-1 activity. Results of our study indicate that meloxicam appears to spare COX-1 activity in vivo, because it had no significant effect on prostaglandin production by the gastric mucosa or thromboxane production in platelets at day 7 or 21 of our study. Conversely, aspirin completely suppressed thromboxane production by platelets and significantly suppressed PGE_2 production in the gastric mucosa after 7 and 21 days of treatment, indicating activity in vivo against COX-1. These findings are consistent with the prediction of previous in vitro data for these drugs, as determined by use of a canine blood assay.[7,19] Data are also consistent with results of other assays for these 2 products in dogs.[6,13] One concern with this model is the potential for prostaglandin formation in response to the gastric biopsy. To minimize this effect, all biopsy specimens were processed within 8 minutes of collection. Any biopsy specimen processed outside this time limit was eliminated from our study. In addition, the original supernatant from each biopsy specimen was discarded. New buffer was added, and the samples were vortexed for 3 minutes to induce prostaglandin synthesis. Therefore, prostaglandin concentrations in these samples are a function of the ability of the sample to produce prostaglandin, and not of baseline prostaglandin concentrations in the sample.

Although COX-1 and COX-2 are involved in the inflammatory response, PGE_2 production by WBC in blood, incubated with bacterial LPS for 24 hours, has been shown to be a function of COX-2 induction.[18] Although no specific data exists for dogs, induction of COX-2 has been found to be the major contributor to prostaglandin production in inflamed joints.[20] Therefore, PGE_2 concentrations in synovial fluid of inflamed joints are most likely the result of COX-2 activity. In our study, PGE_2 concentrations in synovial fluid from inflamed stifle joints, as well as from blood incubated with bacterial LPS, were measured. Aspirin and meloxicam suppressed prostaglandin production in the inflamed synovium after 7 and 21 days of drug administration indicating activity against COX-2 in vivo. Meloxicam also significantly decreased PGE_2 production from LPS-stimulated WBCs at days 7 and 21. However, although a significant decrease in PGE_2 production in LPS-stimulated WBCs was noticed after 7 days of administration of aspirin, a significant rebound occurred at day 21. This apparent reversal in aspirin's activity is unexplainable at this time. Data from our study indicates that meloxicam and aspirin have inhibitory activity against COX-2.

The NSAIDs are starting to be classified on the basis of their selective inhibition of COX-1 or COX-2. This selectivity has traditionally been based on the results of in vitro testing. Although most NSAIDs have a trend toward selectivity of COX-1 or COX-2, substantial differences among study results do exist, especially among species. In our in vivo study, we found that, at commonly prescribed doses, aspirin appears to inhibit COX-1 and COX-2 isoenzymes. Meloxicam appears to selectively inhibit COX-2 and spare COX-1. These findings are consistent with the in vitro and in vivo data for both drugs. Our study is the first, to our knowledge, which confirms the effects of nonselective and selective COX isoenzyme inhibitors, in vivo, on specific target tissues in dogs. The current model provides the first data that allow for the comparison of in vitro and in vivo testing protocols. The use of this model may alleviate concerns about the reliability of in vitro techniques as predictors of NSAIDs selectivity and thus enhance the clinical relevance of these studies.

[a]Boehringer Ingelheim Vetmedica, Inc, St Joseph, Mo.
[b]Schering Plough Animal Health, Union City, NJ.
[c]Abbott Laboratories, North Chicago, Ill.
[d]Cayman Chemical Co, Ann Arbor, Mich.
[e]Amersham Life Science, Buckinghamshire, England.
[f]Sigma Chemical Co, St Louis, Mo.

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